JOURNAL OF DAIRY SCIENCE

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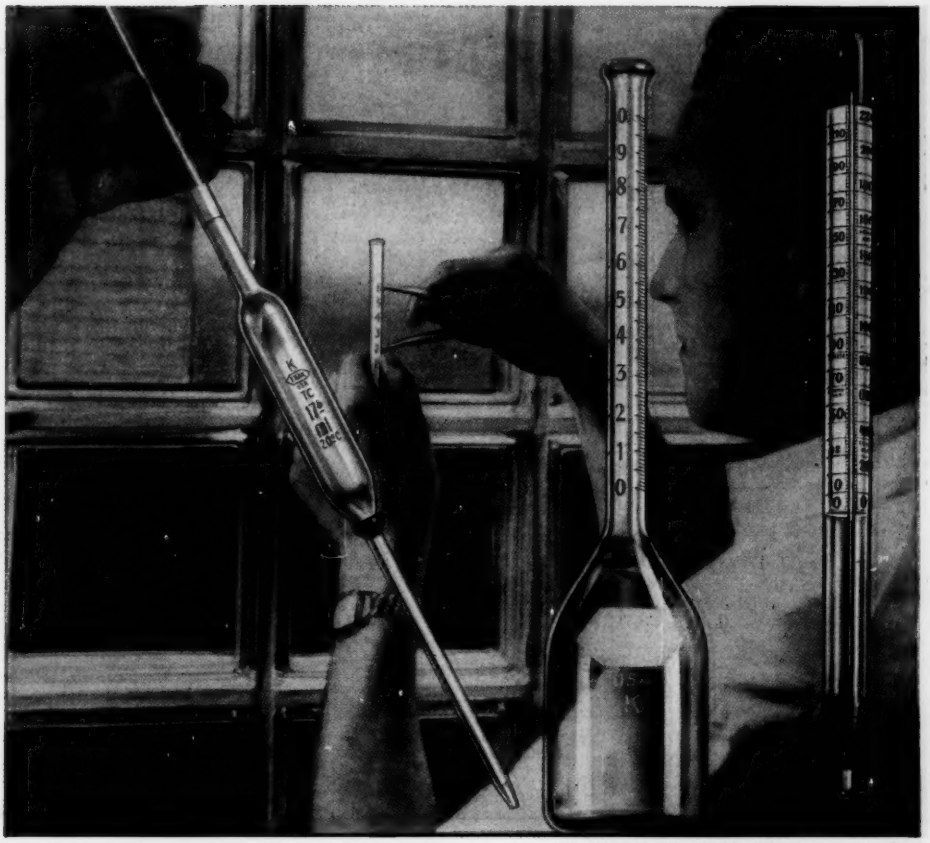

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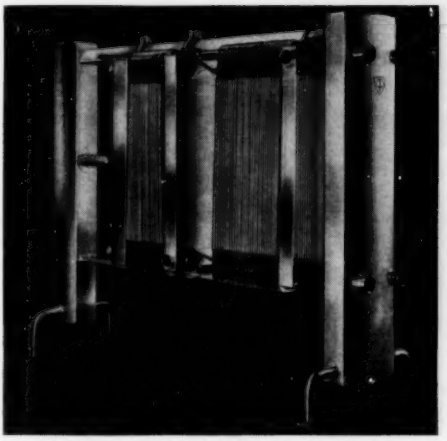

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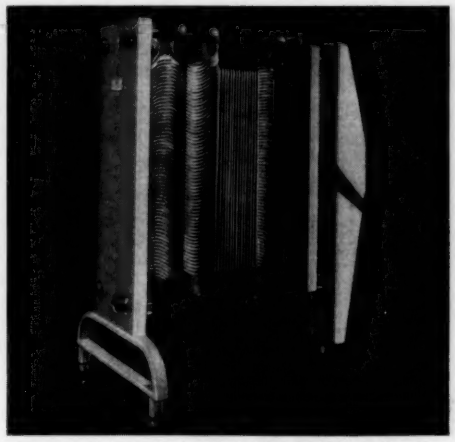

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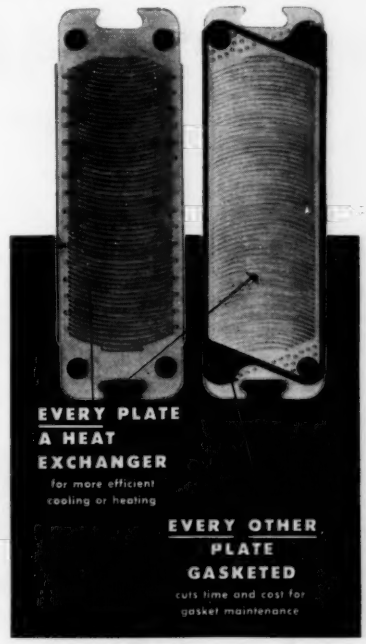

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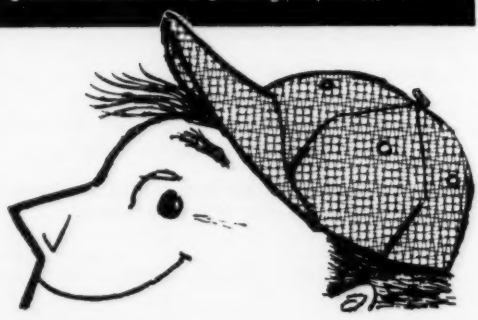

JOURNAL OF DAIRY SCIENCE

VOLUME XXXVIII JULY, 1955 NUMBER 7

SLOW ACID PRODUCTION BY LACTIC CULTURES: A REVIEW[1,2]

F. J. BABEL

Dairy Department, Purdue University, Lafayette, Ind.

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[1] An earlier review (*J. Dairy Sci.*, 26: 83. 1943), prepared by B. W. Hammer and F. J. Babel, describes the organisms present in lactic cultures, the fermentations brought about by them, defects of lactic cultures, etc.

[2] Orders for reprints of this article at $1.00 each will be accepted by the editor until Sept. 1, 1955.

The fermentation of lactose by lactic cultures, resulting in the production of lactic acid, is essential in the manufacture of cultured buttermilk, cultured cream, various cheeses, butter culture, etc. Considerable research has been conducted on the various factors that influence acid production by lactic cultures, indicating that difficulties with slow acid development are encountered wherever such cultures are used. Slow acid production by cultures may vary from slight slowness to complete cessation. When slow acid development is due to several factors, eliminating only one factor may not result in noticeable improvement.

In preparing this review, an attempt has been made to summarize the literature on the factors influencing acid production by lactic cultures and also the literature which suggests methods for decreasing the incidence of slow acid production.

RELATION OF THE MILK SUPPLY TO ACID PRODUCTION

The milk used for propagating lactic cultures, or for manufacture of products in which acid development is essential in preparing a satisfactory product, may affect the rate of acid production. Baker and Hammer *(8)* noted considerable variation in the acidities produced by a culture in lots of milk from individual animals. When milk from individual animals was divided and inoculated with different cultures, the milk from each animal tended to maintain the same general ranking with regard to the acidity produced. Milk with high total solids generally ranked high in developed acidity, but exceptions occurred. Mixed milk from several cows, inoculated with culture, showed less variation in acidity than milk from individual animals. On the basis of bacterial counts and acidity determinations on different samples of milk inoculated with lactic streptococci, Knudsen and Sörensen *(77)* concluded that there was a difference in the nutritive properties of milk samples. Jersey milk and diluted Jersey milk gave greater acid development and higher bacterial counts than did a low-fat milk. Low-fat milk with the addition of 0.005% yeast autolysate was superior to Jersey milk for acid production.

MASTITIS MILK

Milk from infected udders may delay growth of the culture organisms and thereby influence acid production. Davis *(30)* noted that mastitis milk inhibited the culture organisms but that the inhibitory factor in such milk was partially destroyed by pasteurization. Other investigators *(108, 114)* report that heating mastitis milk to pasteurization temperature, or somewhat above, does not improve it materially as a growth medium and that even after heating, such milk may prevent normal acid development by culture organisms. Milk from infected and noninfected quarters of the same cows was used by Prouty *(108)* in studies on the growth of *Streptococcus lactis*. Quarters giving milk with a pH greater than 6.9 did not support growth as well as milk from normal quarters. Addition of 10% mastitis milk to normal milk retarded the growth of *S. lactis*. Whitehead and Cox *(133)* found that lactic streptococci were not able to develop normal quantities of acid in milk containing more than 5,000,000 leucocytes per milliliter.

The influence of the leucocytes was considered to be due to phagocytosis of the streptococci. The inhibitory action of the leucocytes was eliminated by heating the milk (49° to 52° C. for 30 seconds).

RANCID MILK

The milk from certain cows, particularly from those in advanced lactation, sometimes develops a rancid flavor due to the activity of the enzyme lipase. Koestler *(78)* noted that rancid milk inhibited the growth of *S. lactis* and that acid production in such milk was slow. Other investigators *(22, 23, 79, 121, 122)* have confirmed this observation.

Tarassuk *(121)* determined the effect of rancid milk on the rate of coagulation by a small inoculation (0.15%) of lactic culture. In a mixture of 5% rancid milk and 95% normal milk the culture gave a weak coagulum and poor flavor. Coagulation by the culture was delayed 2 hours in a mixture of 20% rancid milk and 80% normal milk, and it was delayed 11 hours when the mixture contained 50%, or more, rancid milk. Increasing the amount of inoculum yielded culture with a better coagulum and flavor. When the culture medium consisted of only rancid milk, even 5% inoculum failed to produce culture of the quality obtained with normal milk. Tarassuk and Smith *(122)* attributed the inhibition of *S. lactis* by rancid milk to the low surface tension brought about by the liberation of free fatty acids from the fat. Growth of *S. lactis* in milk with a low surface tension was inhibited regardless of whether the surface tension was lowered by fatty acids or by addition of a diglycol laurate. Costilow and Speck *(23)* obtained inhibition of *S. lactis* in rancid milk and the inhibition was in direct proportion to the degree of lipolysis. However, lowering the surface tension of normal milk with Tween 40 did not inhibit *S. lactis*. Later experiments *(22)* indicated that the inhibition was due to a toxic effect of the free fatty acids. When fatty acids common to milk-fat were tested for their effect on the growth of *S. lactis*, caprylic, capric, and lauric acids were inhibitory, and the degree of inhibition increased as the concentration of the acid was increased. The effect of myristic acid was not definite, and no other fatty acids tested inhibited the organism.

MILK CONTAINING ANTIBIOTICS

Antibiotics may be present in milk as a result of their use in the treatment of udder infections. The degree of inhibition of a lactic culture in milk containing an antibiotic is related to the type of antibiotic present, its concentration, and the sensitivity of the culture. Katznelson and Hood *(75)* found penicillin to be the most effective antibiotic against lactic acid-producing streptococci. It was 3.3 times as effective as aureomycin and subtilin, 6.6 times as effective as streptomycin, and 33.3 times as effective as bacitracin and chloromycetin.

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penicillin concentrations of 0.5 *(69, 73, 75)*, 0.3 *(6, 124)*, and 0.25 *(81, 117)* units per milliliter; strong inhibition with 0.1 *(69, 75, 81)* unit per milliliter; slight inhibition with 0.1 *(32, 124)*, 0.05 *(6, 117, 124)*, and 0.01 *(53)* unit per milliliter, and no inhibition with 0.05 *(32, 124)* and 0.005 *(53)* unit of penicillin per milliliter. Some cultures are stimulated slightly in milk containing 0.05 unit penicillin per milliliter *(6)*. Hunter *(60)* noted that cultures of *S. cremoris* were inhibited by smaller amounts of penicillin than cultures of *S. lactis*. A given quantity of penicillin in whey appears more effective against lactic cultures than the same concentration in milk *(75)*.

Effect of aureomycin. According to Bell *et al. (9)*, milk obtained from cows 12 hours after treatment with 200 mg. aureomycin per quarter markedly inhibited culture growth; even the milk obtained 60 hours after treatment showed some inhibition of culture activity. Autoclaving milk at 15 lb. steam pressure for 10 minutes did not destroy the aureomycin in it. Bradfield *et al. (12)* determined the rate of acid production by lactic cultures in milk obtained from quarters treated with 213 mg. aureomycin. Practically no acid developed in the milk obtained for four milkings after treatment, and the milk gave some culture inhibition until the eighth milking. When aureomycin was added to milk, cultures were inhibited by 0.1γ but not by 0.01γ per milliliter. Krienke *(82)* obtained nearly normal acid production by lactic cultures in milk containing 0.05γ aureomycin per milliliter; cultures were inhibited by 0.5γ per milliliter. The author calculated that the aureomycin from a single infusion (200 mg.) would be sufficient to inhibit acid production almost completely in 1,000 lb. milk. Some cultures tested by Johnstone *(72)* showed limited resistance to aureomycin.

Extent of antibiotics in milk. Data obtained by Bryan *(13)* indicate that milk from cows treated with antibiotics is not being withheld from the milk supply. Of 27 samples of herd milk selected at random in the Lansing, Mich., area, one contained no penicillin, one contained 0.50 unit, five contained 0.25 unit, seven contained 0.12 unit, ten contained 0.06 unit, and three contained 0.03 unit penicillin per milliliter. Kosikowsky *et al. (80)* analyzed 1,794 samples of blended pasteurized milk obtained throughout New York state and found inhibitory substances in about 7% of them. However, only about 1% strongly inhibited lactic cultures. Samples obtained during the late summer had a smaller incidence of inhibitory materials than those obtained in the early spring. Johns *(70)* determined the extent of inhibitory substances in milk from the district of Ottawa. Of 344 samples tested in the spring, seven greatly inhibited lactic culture and 18 gave mild to medium inhibition. Of 298 samples tested in the summer, four greatly inhibited culture and 12 gave mild to medium inhibition.

Methods for decreasing the incidence of culture failures due to antibiotics. Several methods have been studied for counteracting the effect of antibiotics in milk on lactic cultures. Most of the studies have involved penicillin, but certain of the recommendations apply to other antibiotics as well.

Withholding milk from animals treated with antibiotics. The most satisfactory method for eliminating culture failures caused by antibiotics in milk would be to withhold milk from the treated animals for several milkings. The number

of milkings after treatment that should be withheld depends upon the amount of antibiotic used, the type of carrier (water, ointment, etc.), and the amount of milk secreted by the animal. Watts and McLeod *(130)* injected 119 cows with 20,000 units of penicillin in 50 ml. distilled water per quarter. The average total recovery in milk 12 hours after injection was 3,800 units for ten cows yielding 5 lb. per milking and 55,100 units for eight cows yielding 20 lb. per milking. Kastli *(74)* concluded that milk from penicillin-treated cows should not be used for processing until the fifth day following treatment since milk from individual quarters treated with 20,000 units of penicillin contained 3 to 4.5 units per milliliter 12 hours after treatment and 0.2 to 0.4 unit per milliliter 24 hours after treatment. Trussell and Stevenson *(125)* found that foremilk and strippings contained 5.4 and 2.8 units penicillin per milliliter, respectively, when an animal was treated with bougies containing 25,000 units and was milked 12 hours after treatment. Hunter *(61)* determined the concentration of penicillin in milk from quarters given four or five successive infusions of 17,000 to 20,000 units of penicillin in distilled water. The first milking after treatment contained 1.3 to 5.0 units and the second milking 0.1 to 0.5 unit per milliliter. There was no substantial build-up as a result of successive treatments. He considered it unnecessary to exclude milk beyond two milkings. Hansen *et al. (46)* noted that the pooled milk from four cows, each treated in one quarter with 200,000 units of penicillin in 40 ml. of 1% sulfamerazine, failed to develop sufficient acidity for cheese making until five or six milkings after treatment. Almost identical results were obtained with penicillin alone. There was no diffusion of penicillin from one quarter to another. Jackson and Bryan *(67)* determined penicillin levels in milk after administration of various penicillin products and dosages to cows in mid-lactation. Penicillin in water or penicillin bougies administered in dosages of 25,000, 50,000, and 100,000 units could not be detected in milk after 2.5, 3, and 4 days, respectively. Penicillin in ointment base, used at the rate of 50,000 and 100,000 units, could be detected after 4 days but not after 5 days. Procaine penicillin in oil, used at the rate of 300,000 units, could be detected in milk 9 days after use. Jørgensen *(73)* also noted that the concentration of penicillin in milk was related to the amount injected and that duration in the udder depended upon the type of compound used. Jepsen and Overby *(69)* determined the rate of acid development in milk obtained from a herd being treated with bougies (25,000 units). During treatment, the inclusion of 3.3% of milk from treated animals with the normal milk delayed acid production, and 10% gave practically no acid development. Forty-eight hours after the last treatment equal parts of milk from treated and untreated animals could be mixed with practically no effect on acid production. Dumais *(32)* treated cows with seven commercial brands of penicillin, using 100,000 units per treatment. Negative tests for penicillin were obtained 3.5 to 6 days after administration, depending upon the brand used.

Penicillinase. The enzyme inhibitor for penicillin is called penicillinase or penase. Hood and Katznelson *(53)* found that 0.2γ penicillinase per milliliter exerted a marked effect on penicillin in a concentration of 1 unit per milliliter

and completely inactivated 0.05 to 0.1 unit per milliliter. Krienke *(82)* did not consider it practical to inactivate penicillin in milk with penicillinase. The cost of treating milk containing 1 unit penicillin per milliliter with penicillinase exceeded the cost of the milk. Auclair *(3)* considered it practical to use penicillinase in the production of Gruyère cheese. Under commercial conditions, 1 unit of penicillin per milliliter was inactivated by 0.05 ml. penicillinase acting at 14° C. and added to milk 16 hours before the lactic culture or by 1 ml. of penicillinase added just before the culture. From milk, Czekalowski *(26)* isolated lactobacilli, *Chromobacterium violaceum*, and some staphylococci which produced endocellular penicillinase. *Bacillus subtilis* produced both the endo- and exo-cellular enzyme. Optima for penicillinase were 37° C. and pH 7.0. Jepsen and Overby *(69)* noted that penicillinase was produced by certain coliform organisms and some species of the genus *Bacillus*. Complete inactivation of 1 unit penicillin per milliliter required 0.5 unit penicillinase per milliliter; the time required for inactivation varied with the temperature from 30 minutes at 30° C. to 120 to 150 minutes at 8° C. Two types of penicillinase studied by Jensen and Pedersen *(68)* were thermostable.

Chemicals. Opening the β-lactan ring in the penicillin molecule with formation of penicillinoic acid results in complete loss of its biological activity *(69)*. Such a reaction occurs by addition of various acids, alkalies, ammonia, and amines. Hydroxylamine is very reactive; 0.005% completely inactivates 1 unit penicillin in 1 hour. However, hydroxylamine inhibits streptococci and is toxic. Cysteine does not inhibit penicillin when added at the rate of 0.01 to 0.1 mg. per milliliter *(53)*.

Reconstituted milk. The use of reconstituted milk for avoiding difficulties resulting from the unexpected presence of penicillin in the fresh milk supply was recommended by Scott *(117)*. Previously, Horrall *et al. (55)* noted that lactic cultures propagated in reconstituted skimmilk were more uniform in activity from day to day than cultures propagated in selected herd milk. However, it was necessary to check each lot of nonfat dry-milk solids to be certain that it would yield cultures having satisfactory activity. The water used for reconstitution also affected culture activity; distilled water was always satisfactory.

Increase in inoculum. Krienke *(83)* suggested an additional inoculation of cheese milk with culture when slow acid production was evident. This suggestion is based on the findings of Maass and Johnson *(86)*, who noted that each bacterial cell is capable of binding several molecules of penicillin and such bound penicillin remains bound during subsequent multiplication of the cells in a penicillin-free medium. Bacterial cells added to milk containing penicillin may adsorb considerable penicillin so that acid production does not progress at a normal rate. If more cells are added, there will be less penicillin to adsorb and the cells added later may function normally. Jensen and Pedersen *(68)* propagated lactic cultures in milk containing not more than 0.1 unit penicillin per milliliter by using an increased inoculum; the procedure was not effective with higher concentrations of penicillin.

Adaptation of cultures. Katznelson and Hood *(75)* attempted to adapt six cultures to penicillin. Only one culture could be adapted, and it coagulated milk containing 2.1 units penicillin per milliliter. The adapted culture remained resistant to penicillin after more than 20 transfers in penicillin-free milk. Trembath *(124)* as well as Jepsen and Overby *(69)* developed penicillin resistance in certain cultures. In one adapted culture, the *Leuconostoc* strain disappeared at a penicillin concentration of 0.25 unit per milliliter and no biacetyl or acetylmethylcarbinol was formed by the culture *(69)*.

Legislation. The Federal Security Agency, Food and Drug Administration, has concluded that the direct or indirect addition of antibiotics to foods for human consumption constitutes a public health hazard *(35)*. The reason for this conclusion is that food so treated may cause sensitization of the consumer to such antibiotics and may result in the emergence of strains of pathogens resistant to these drugs. Foods for human consumption are considered adulterated if they contain antibiotics.

MILK CONTAINING SULFA COMPOUNDS AND BACTERICIDES

Sulfa compounds may be present in milk because of their use, either alone or in combination with antibiotics, in the treatment of udder infections. Chlorine compounds, iodophors, and quaternary ammonium compounds are used as bactericides and may be present in milk because of failure to drain utensils treated with them or because of wilful addition.

Sulfa compounds. According to Krienke *(82)*, 5 ml. of a 25% solution of sulfamethazine added to 100 ml. of milk practically prevented acid development by lactic culture; acid development was inhibited slightly by 0.1 ml. of the 25% solution in 100 ml. milk. No sulfa drugs were detected in 922 samples of milk tested specifically for them by Kosikowsky *et al. (80)*.

Chlorine compounds. Calbert *(14)* noted that acid production by a lactic culture in sterile skimmilk was retarded by 25 to 200 p.p.m. of chlorine. Miller and Elliker *(91)* did not obtain acid development in milk containing 100 to 200 p.p.m. chlorine when it was inoculated with a commercial lactic culture or *S. cremoris* and incubated at 37.8° C. With incubation at 21.1° C., the cultures were not inhibited completely by 200 p.p.m. Babel *(7)* reported that acid production by lactic cultures was slightly inhibited by 5 p.p.m. available chlorine in milk and greatly inhibited by 25 p.p.m., added as hypochlorite.

Iodophors. An iodophor added to milk in amounts ranging from 5 to 50 p.p.m. had no effect on acid production by lactic cultures; small quantities of the bactericide actually stimulated acid development *(7)*.

Quaternary ammonium compounds. Miller and Elliker *(91)* noted that acid production by a commercial lactic culture or an *S. cremoris* culture was inhibited slightly in milk containing 5 p.p.m. of a quaternary or detergent-sanitizer containing a quaternary. These cultures were inhibited almost completely in milk containing 25 to 30 p.p.m. of quaternary when incubated at 37.8° C.; with incubation at 21.1° C., 50 to 75 p.p.m. of quaternary almost completely inhibited the cultures. In actual cheese manufacture, 5 p.p.m. of quaternary in the milk

delayed milling time about 15 minutes, 10 p.p.m. gave a delay of 45 to 60 minutes, and 20 p.p.m. gave a whey acidity of only 0.20% after 7.5 hours. Babel *(7)* obtained a definite decrease in rate of acid production by lactic cultures grown in milk containing 3 p.p.m. of quaternary and no acid production in milk containing 50 to 100 p.p.m. Curry and Barber *(25)* found that a detergent-sanitizer containing a quaternary was more effective against *S. lactis* than the quaternary used alone. Milk containing 2.5 p.p.m. or less of the detergent-sanitizer had little or no effect on acid production. *S. lactis* did not develop acid in milk containing 50 p.p.m. of quaternary. Concentrations of quaternary up to 25 p.p.m. did not interfere with growth and multiplication of cultures but did block an enzyme system essential in the formation of lactic acid. *Leuconostoc citrovorum* was more resistant to the detergent-sanitizer than *S. lactis*. Lindgren *(85)* noted that 10 p.p.m. of a quaternary in milk retarded acid and aroma production by lactic culture. With repeated inoculation into milk containing a quaternary, the culture quickly adapted itself, and aroma production returned to normal.

FACTORS ASSOCIATED WITH CULTURE PROPAGATION THAT INFLUENCE ACID PRODUCTION

In the propagation of lactic cultures, factors other than the milk supply may influence acid production by the mother culture or succeeding propagations. The culture, heat treatment given to milk, incubation temperature, incubation time, air supply, etc., influence acid development.

THE CULTURE

The lactic acid streptococci in cultures show considerable variation in acid production. Hammer and Baker *(44)* suggested a division of the *S. lactis* group into varieties. One of the divisions was based on the rate of coagulation, and organisms coagulating milk slowly were designated as *S. lactis* variety *tardus*. When a culture which coagulated milk rapidly was plated and colonies picked into litmus milk, Harriman and Hammer *(47)* obtained strains of *S. lactis* that coagulated milk both rapidly and slowly. Strains isolated from a slow culture were all slow in their rate of coagulation. Hunter *(57)* investigated variability among strains of *S. cremoris*. One strain showed a definite change in acid production as well as a change in colony form. Other investigations *(29, 33, 99, 128)* have indicated variation among cultures with respect to acid production. Variation in lactic acid production by cultures is recognized by companies distributing them, and frequently the plant operator is given a choice of cultures based on the rate of acid production.

HEAT TREATMENT OF MILK

The heat treatment given milk intended for culture propagation, preparation of bulk culture, or for manufacture of products in which culture is employed, influences the rate of acid production. Hammer and Baker *(45)* noted variations in the rate of coagulation by cultures inoculated into milk that was given differ-

ent heat treatments. Milk heated at 62.8° C. for 30 minutes developed acid and coagulated more slowly than milk heated to considerably higher temperatures. Essentially the same rate of growth of the culture organisms occurred after heating at 71.1° C. for 30 minutes as when a higher temperature was used for this period. With a holding time of 30 minutes, the influence of heat seemed to be gradual and spread over a considerable temperature range instead of occurring at a definite temperature. Rice *(113)* noted that the lactic acid streptococci of cultures formed acid almost as rapidly in milk heated momentarily at 76.6° C. as at higher temperatures for longer times but that there was a slower rate of fermentation in milk heated at 62.8° C. for 30 minutes and also in unheated fresh milk. Davis *(30)* obtained less acid development in raw milk inoculated with culture than in milk heated at 73.9° C. for 30 minutes, and milk boiled for 24 hours formed more acid than milk heated to 82.2° C. by the flash method. Babel *(6)* determined the rate of acid production by two lactic cultures in raw milk, milk heated at 62.8°, 71.1°, and 85° C. for 30 minutes, and milk autoclaved at 15 lb. steam pressure for 15 and 25 minutes. With both cultures, acid production was slowest in raw milk and only slightly greater in milk heated at 62.8° C. for 30 minutes. Holding the milk at 85° C. for 30 minutes improved acid production in it by lactic cultures in comparison to raw milk or milk heated at 62.8° C. for 30 minutes, but this exposure was not as satisfactory as autoclaved milk or milk heated at 71.1° C. for 30 minutes. Milk autoclaved at 15 lb. steam pressure for 15 minutes was the most satisfactory exposure from the standpoint of acid production of those tested. One culture produced acid more rapidly in milk autoclaved at 15 lb. steam pressure for 25 minutes than in milk heated at 71.1° C. for 30 minutes; the reverse was true with the other culture.

Using milk from individual cows, Foster *(37)* obtained greater acid production by *S. lactis* in autoclaved milk (15 minutes at 120° C.) than in milk heated at 80° C. for 10 minutes. Herd milk and mixed herd milk gave similar results. When mixed milk was heated at 80° C. for various times, acid production by an *S. lactis* culture was increased with an increase in the exposure time (10 to 300 minutes). Acid was produced more rapidly in milk heated to 100° C. for various times than when heated to 80° C. for similar periods. With exposure at 100° C., the rate of acid production was increased more by increasing the holding time in the range of 10 to 120 minutes than in the range of 120 to 240 minutes. Autoclaving mixed milk at 120° C. for periods up to 15 or 20 minutes gave increases in acid production by *S. lactis,* and the increases were greater with extended holding times; periods greater than 15 or 20 minutes gave decreased acid production, and the decreases were greater with extended holding times. The increase in acid production as a result of the heat treatment was considered due to a partial hydrolysis of casein which provided the organisms with increased amounts of usable nitrogen.

Singh and Laxminarayana *(119)* found that heating raw milk to 62.8° C. and holding for 30 minutes improved the growth and activity of lactic cultures; however, milk heated to boiling and then cooled was even more satisfactory. They were of the opinion that some heat-stable antibacterial substances were present

in raw milk and to a greater extent in early lactation, late lactation, and mastitis milk in increasing order. Early lactation milk was improved considerably by heat treatment and very nearly approached normal milk in acid production. Although heating improved acid development in mastitis milk and advanced lactation milk, such milk was inferior to normal milk.

INCUBATION TEMPERATURE

Although mother cultures and bulk culture commonly are incubated at 21.1° to 22.2° C. to maintain a proper balance between the citric acid-fermenting and lactic acid-producing streptococci, these temperatures are not the most favorable for rapid acid development. Consequently, higher temperatures are used in some manufacturing operations where rapid acid development is advantageous. Studies on three lactic cultures by Golding *et al. (39)* indicated that maximum acid development in 8 hours took place between 29.4° and 32.2° C.; development of acid at 25° and 35° C. was approximately one-half that at the optimum, and at 15.5° and 37.7° C. it was insignificant during the 8-hour test. There was considerable difference in the rates of acid development by the three cultures. Harrison and Dearden *(48)* determined the rate of acid production by three cultures at temperatures of 22°, 28°, 30°, 37°, 40°, and 45° C. Culture S produced acid at temperatures up to 37° C. but failed to produce acid at 40° C. Cultures X and Y produced acid at 40° C. All of the cultures failed to grow at 45° C. After incubation at 40° C. for 24 hours, culture S was incapable of further growth at either 22° or 28° C. Cultures X and Y initiated growth after a similar incubation.

Dahlberg and Ferris *(28)* inoculated milk with cheese culture and held one portion at 30° and another at 37.7° C. Acid development at 30° C. was rapid, but at 37.7° C. it was very slow. When milk inoculated with culture was held at 30° C. for 2 hours followed by incubation at 37.7° C., acid development was quite rapid. The rate of acid production by seven lactic cultures grown in reconstituted skimmilk at 30°, 36.6°, 38.4°, and 40° C. was determined by Babel *(4)*. Four cultures produced acid most rapidly at 36.6° C. and three cultures most rapidly at 30° C. With all cultures there was a marked difference between acid production at 30° or 36.6° C. and 38.4° C., cultures held at 38.4° C. being slower in acid production. Most cultures produced acid very slowly at 40° C. When cultures held at 30° C. for 7 hours were compared with cultures held 2 hours at 30° C., then 2 hours at 40° C., and 3 hours at 30° C., six of the seven cultures tested produced acid more rapidly when held continuously at 30° C. Golding *et al. (41)* found that propagation of cultures at 21.1° to 22.2° C. gave a more active culture than propagation at 30° C.

INCUBATION TIME

The usual incubation time for cultures held at 21.1° to 22.2° C. is approximately 14 to 16 hours, but a slight increase in incubation time apparently does not influence culture activity. Hammer and Baker *(45)* noted that holding cul-

tures at 21.1° C. for 30 hours after coagulation did not appreciably increase the time required for the cultures to coagulate milk. Golding *et al. (39)* reported that the acidity of fresh cultures, as usually found in cheese factories, had little relation to their rate of acid development in milk. Also, culture held cold for 24 hours did not change the activity of it when grown previously at the approximate optimum temperature. Johns and Berard *(71)* found that increasing the incubation time of a culture for a period greater than usually encountered in cheese-factory practice failed to decrease the rate of acid development, or to lower the final acidity attained by the culture. An over-ripened portion of a culture produced acid faster during cheese manufacture than a normally ripened portion. With two of the three cultures studied, the over-ripened portion contained more rapid-coagulating organisms than the normally ripened portion. After 30 days of repeated over-ripening, the flavor of the over-ripened portions was superior to that of the normally ripened portions. Babel *(6)* compared the rate of acid development by cultures incubated at 22.2° C. for 14 to 16 hours and cultures incubated at 30° C. for 8 hours and then held cold (7.2° C.) for 18 hours. There was no appreciable difference between the two methods of preparing cultures so far as acid production was concerned.

AIR SUPPLY

Milk inoculated with culture commonly coagulates first at the bottom of the container. Toens and Hammer *(123)* compared the rate of acid development by cultures in thin layers of milk held in cotton-stoppered flasks with the rate in deeper layers held in glass-stoppered bottles. Acid development was more rapid with a restricted than with an abundant air supply. Rice *(113)* noted that aeration of milk retarded development of acid by lactic acid streptococci. Katznelson and Hood *(76)* determined the rate of acid production by five cultures in agitated and nonagitated milk. Three cultures produced less acid in agitated milk, but this effect could be overcome by the addition of glutathione; the other two cultures were not affected appreciably by agitation. Raw, unagitated milk was not affected by glutathione, indicating a satisfactory reducing condition for growth and acid production by the culture organisms. When raw milk was agitated and exposed to the atmosphere, it became a much less favorable medium for the culture organisms; addition of glutathione to such milk improved it as a culture medium. Freshly pasteurized unagitated milk produced the same amount of acid with or without glutathione, but a lot of milk treated similarly except that it was agitated gave a definite response to glutathione. Golding *et al. (41)* found that aeration of whole milk before inoculation with culture did not affect its activity appreciably. However, agitation during incubation increased acid development with two of three cultures tested. The effect was apparently associated with the creaming of the milk, since it did not occur with skimmilk.

BACTERIOPHAGE

Bacteriophage (phage) has been defined as a particulate entity of submicroscopic size which increases in numbers only in the presence of living susceptible

bacterial cells and usually causes the lysis or disruption of the bacterial cells *(94)*. The typical interaction between phage and bacterium consists of adsorption of phage by the bacterium, followed after an interval by lysis of the bacterium and a many-fold increase in the number of phage particles *(140)*.

Many instances of slow acid production by lactic cultures have been due to the action of phage. The increased amount of trouble now encountered with phage is attributed to: *(a)* ability to recognize the source of the trouble, *(b)* more extensive use of pasteurization, which destroys the normal natural lactic acid streptococci that tend to be resistant to phage activity and that could carry on when the susceptible culture was lysed, and *(c)* dissemination of phage by certain cultures distributed to manufacturing plants *(94)*.

SIZE OF BACTERIOPHAGE PARTICLES

Bacteriophage active against *S. lactis* appear sperm-shaped. Because of their small size, phage particles generally are observed with the electron microscope, although they have been seen with a regular microscope by using special techniques, and with the phase microscope *(52)*. Cherry and Watson *(15)* give the following dimensions of phage particles: diameter of head structure 73 mμ, length of tail 160 mμ, width of tail 20 mμ, and over-all length 230 mμ. Parmelee *et al.* *(104)* found that phage active against *S. lactis* had a total length of 180 to 280 mμ, the heads were spherical and 60 to 90 mμ in diameter, and the tails were 20 to 40 mμ wide and 120 to 190 mμ long. Williamson and Bertaud *(143)* report the isolation of one phage having a spherical head 70 to 80 mμ in diameter attached to a long flagellum-like tail 15 mμ wide and 560 to 610 mμ long. Nine other phages studied by them had dimensions similar to those reported by other investigators.

ENUMERATION OF BACTERIOPHAGE

The number of bacteriophage particles in a bacteria-free filtrate may be estimated by adding serial dilutions of the filtrate to a sensitive bacterial culture or by counting plaques on a solid or semi-solid medium. Shew *(118)* noted that when two drops of skimmilk culture were spread over the surface of plates poured with yeast-lactose-phosphate agar, maximum plaque formation occurred in the area where the drops were placed. Addition of 0.5 ml. of cell-free milk serum to 15 ml. agar resulted in plaque formation over the entire plate. The addition of calcium chloride had the same effect as serum, the optimum amount (0.02 to 0.007 *M*) varying with the phosphate content of the medium. Yen *(145)* considered that phage particles and organisms would be more uniformly distributed in a single layer of agar medium than in a smear of a similar mixture over the surface of a solid medium. However, he experienced difficulty in obtaining clearly defined plaques at different levels in the agar layer. Gratia *(42)* overcame this difficulty by mixing a small amount (0.5 to 1.0 ml.) of a phage suspension with 3 ml. of 0.7% melted agar medium and a small amount of bacterial cells. The mixture was poured over the surface of plates containing 20 ml. of solidified 1.5% agar medium and spread evenly in a thin layer. With this

method, plaques were formed in a thin layer of medium resting on an uninoculated nutrient base. A cell concentration of 2 to 4 $\times$ 10^7 per milliliter of plated mixture was satisfactory.

Cherry and Watson *(15)* noted that the amount of inoculum influenced the size and number of plaques. Plaque size decreased when the cell count was less than 1.3 $\times$ 10^6. Turner *(126)* studied various factors which influence plaque counts when using a two-layer plate technique. For enumerating phage active against *S. lactis*, a base layer of 12 ml. tomato juice–peptonized milk medium containing 1% agar and an overlay of a mixture of 1 ml. *S. lactis* cells, 1 ml. of phage suspension, and 1 ml. of tomato juice–peptonized milk medium containing 1.5% agar was most suitable. The plating medium was adjusted to pH 5.8 to 6.0. Increasing the agar concentration in the overlay to 0.6 and 0.67% decreased the plaque count to 57 and 46%, respectively, of the count obtained with an agar concentration of 0.5% *(127)*. The greatest number of plaques were formed with 21 to 35 million bacteria in the overlay. Milk, rather than water, as the diluting medium also gave higher plaque counts. With the two-layer technique, counts were slightly higher than the most probable number calculated from the limiting dilution method. Potter and Nelson *(106)* obtained higher plaque counts by use of a 2.5% calcium chloride solution as a cell diluent in the two-layer technique. Old phage preparations were stimulated by the calcium chloride to a greater extent than fresh preparations. Also, they noted that phage multiplication did not occur in a calcium deficient medium that supported growth of lactic streptococci *(107)*. The amount of calcium added as $CaCl_2 \cdot 2H_2O$ necessary for phage proliferation varied with the phage-cell combination from 6.7 $\times$ $10^{-5}M$ to 5.4 $\times$ $10^{-4}M$.

MODE OF BACTERIOPHAGE ACTION

In 1933, it was reported that addition of slow-coagulating lactic cultures to pasteurized or sterilized milk often caused considerable delay in coagulation of the milk by normal cultures; the restraining agent passed through a bacteria-removing filter and was inactivated by boiling *(66)*. Nelson *et al. (93)* noted that when bacteria-free filtrates from cultures were added to freshly inoculated *S. lactis* cultures or regular lactic cultures, a majority of the filtrates from slow cultures and a smaller percentage from apparently normal cultures markedly restrained acid production and multiplication of organisms. Bacteria-free filtrates from slow and from normal cultures varied widely in their ability to inhibit different strains of *S. lactis*.

Observations by Hunter *(59)* indicated that phage active aganist *S. cremoris* was quite strain specific but that phage active against *S. lactis* usually had a wide range of lytic activity. Overby *(100)* isolated phage that was relatively non-specific from cultures that had failed repeatedly to produce acid. The phage inhibited 11 of 14 strains of lactic acid bacteria isolated from several multiple-strain cultures. By means of single plaque isolations, Babel *(5)* obtained four phage types from the whey of a vat of Cheddar cheese in which a multiple-strain lactic culture failed to produce acid. The phage types were active against 12

of 15 *S. lactis* cultures picked from plates inoculated with the mother culture. One phage type was active against only one *S. lactis* culture and the others were active against two, six, and nine cultures. Deane and Nelson *(31)* found that unpurified filtrates of whey and culture from plants experiencing slow acid production contained phage active against four to ten of 13 test cultures. They concluded that when a plant using a multiple-strain culture experiences slow acid development due to phage, either more than one strain has been lysed or the culture contains one predominant strain.

By mixing unrelated phage-sensitive strains of lactic streptococci and using the homologous phage to eliminate each strain successively from the mixed culture, Nichols and Ineson *(96)* thought it might be possible to study the behavior of strains in a multiple-strain culture and explain culture recovery. Activity tests showed that in each culture mixture, one strain became prominent on prolonged daily transfer. Strain predominance could be influenced by the temperature of incubation. A study of the behavior of strains was complicated by the presence of secondary-growth organisms and natural variants. Hunter *(62)* noted that when *S. cremoris* (HP) was used in cheese manufacture, it was always lysed by a specific phage race even though several phage races were capable of lysing the culture. Laboratory and field results suggested that the phage race active against culture HP had a special affinity for the organism and that its presence interfered with the growth of other phage races which could lyse the organism in its absence. Reiter *(112)* grew four strains of lactic streptococci separately and inoculated them into one lot of milk for use as bulk culture. Phage which lysed one culture (HP) was detected often in the bulk culture. Tests indicated that culture HP was an indicator strain and the other three were lysogenic. Subcultures of the three lysogenic strains, after replating and picking colonies, continued to lyse culture HP.

Five phage strains studied by Turner *(126)* proliferated by a step-wise increase, the probable burst time being about 65 minutes and the average burst size about 90 particles. The proliferation curve for each phage showed lag, logarithmic and resting phases. The optimum rate of multiplication for both phage and bacteria was 32° C. Collins *(18)* noted that the addition to skimmilk of 5×10^{-3} or 5×10^{-4} phage particles per milliliter and 0.5% of milk cultures of host bacteria gave normal acid development with two cultures. However, in the absence of evident phage action, the phage titer increased to 10^7 in 5 hours. With addition of 4.5×10^{-1} and 2.5×10^{-2} phage particles per milliliter, and the same amount of host bacteria, mass lysis occurred. One *S. lactis* culture did not undergo mass lysis when 4% was added to milk containing 25 phage particles per milliliter but four other cultures required 8% inoculum to prevent mass lysis. Mass lysis did not occur in milk if coagulation occurred before the phage concentration was sufficient to cause mass lysis.

Certain strains of lactic streptococci studied by Hunter *(59)* formed plaques on solid media with no evidence of phage activity in milk. Phage concentrations of 10^4 or 10^5 particles per milliliter developed with no adverse effect on the organisms, and such titers were maintained with daily subculture for long peri-

ods. The cultures varied in their ability to retain phage, the retention period ranging from 1 to 12 weeks. The presence of phage in a culture sometimes inhibited the action of a second phage race which otherwise would lyse the culture. Ford and Babel *(36)* added phage to a culture of *S. lactis* and noted that the secondary-growth organisms retained phage for 132 transfers with incubation at 21° C. and for 10 transfers with incubation at 37° C. The culture was resistant to other phages as long as it retained the type which originally caused lysis. Czulak *(27)* also noted that lysogenic strains of *S. lactis* were protected from phage. However, if for some reason a high phage titer was reached, the culture underwent mass lysis. Nelson and Parmelee *(94)* detected phage in several commercial cultures. Such cultures appeared normal and had normal activity.

FACTORS INFLUENCING BACTERIOPHAGE MULTIPLICATION

Bacteriophage multiplication is influenced by some of the same factors which influence bacterial growth.

Cherry and Watson *(16)* found that adsorption of bacteriophage by *S. lactis* was about three times as great in a medium consisting of tryptone, yeast extract, and glucose as in the same medium when tryptone was omitted. Electrolytes stimulated both cellular lysis and virus adsorption at concentrations ranging from 0.005 to 0.05 *M*. The average yield of virus per infected cell in tryptone yeast-extract medium at pH 7 and 30° C. was about 70 plaque-forming particles. The burst size was reduced by one-third when tryptone was omitted from the medium. Shew *(118)* obtained incomplete lysis of organisms in a yeast-extract lactose phosphate broth unless milk serum was added. Addition of calcium chloride had the same effect as serum, the optimum amount varying with the phosphate content of the medium. Collins *et al. (21)* noted that the omission of individual amino acids, purine and pyrimidine bases, and vitamins from a defined medium affected the multiplication of bacteria and phages of two *S. lactis*-phage combinations. Eight phages did not multiply on their hosts in the defined medium. When calcium was available, the defined medium supported multiplication of phage. Collins *(17)* also found that phage did not multiply in Niven's medium when enriched with sodium acetate and Tween 80 even though it supported growth of *S. lactis* and *S. cremoris*. All of the phages multiplied when calcium was added to the medium. Six strains of phage that were unable to multiply in a calcium-deficient medium in which their host bacteria grew well were studied by Potter and Nelson *(105)*. Maximum virus production occurred with 0.004 to 0.03% calcium but slight changes in calcium content affected the phage titer. The absence of calcium did not affect adsorption of phage on the host bacteria.

The influence of the pH of the medium on lysis of *S. lactis* by phage was determined by Cherry and Watson *(15)*. At pH 4, the cells were not influenced by phage, or infection did not prevent some cellular multiplication. At pH 5, some cells were probably infected, since incomplete lysis occurred. Cells were completely lysed at pH 6, 7, and 8, but at pH 8 the latent period was extended. Overcast *et al. (101)* found the optimum pH for phage production to be about

6.5. The five phage strains studied multiplied quite well in the range of pH 5.4 to 7.5. One phage multiplied over the range pH 4.8 to 9.4 and another multiplied in the range 5.2 to 7.6. Under optimum conditions, such as pH 6.5, a temperature of 30° C., and a milk medium, an initial inoculum of 10 phage particles per milliliter may increase to as many as one million per milliliter in 5 hours or 200 million per milliliter in 7 hours *(94)*.

Zehren and Whitehead *(146)* noted that certain lactic cultures could not be used in cheese manufacture for more than 1 or 2 days in succession without failure to produce acid even though all practical means were taken to exclude phage contamination; other cultures were less likely to fail and could be used for longer periods. The duration of usefulness of a culture was related to the extent of phage in the air, and the extent of phage in the air was related to the concentration in the whey. Two characteristics of phage were related to the concentration in whey: *(a)* the latent period or time from infection of the bacterial cell to lysis, and *(b)* its burst size or average yield per infected bacterial cell. Among six organism-phage combinations, the latent period varied from 40 to 90 minutes at 30° C. and the burst size from 21 to 77 phage particles. By studying the action of different phages on one bacterial strain, it was shown that both latent period and burst size were characteristics of the phage and not of the bacteria.

DESTRUCTION OF BACTERIOPHAGE

Effect of heat. When trials were conducted at approximately neutral reaction, Nelson *et al. (93)* noted that some strains of inhibitory principle survived 70° C. for 10 minutes but not for 15 minutes; other strains were inactivated by less heat. The principle was more resistant to heat than its homologous organism. Nine phage strains tested by Whitehead and Hunter *(136)* had thermal death points in the range of 70° to 75° C. for 30 minutes at pH 6. According to Cherry and Watson *(15)*, very short exposures to temperatures below 55° C. had a negligible effect on phage. The rapid rate of inactivation between 55° and 65° C. indicated that an important mechanism of the virus was being destroyed. Phage studied by Miklik *(90)* could withstand 60° C. Heating rapidly to 80° C. or holding at 70° C. for 30 minutes weakened phage considerably, and heating to 90° C. or holding at 80° C. for 30 minutes inactivated the phage. At pH 8, Novák *(98)* found that phage was destroyed by holding at 100° C. for 5 seconds; at 95° C. for 1 minute; at 85° C. for 4 minutes; at 75° C. for 20 minutes; and at 65° C. for 30 minutes. At pH 6, phage was destroyed almost instantly at 100° C.; in 10 seconds at 95° C.; in 1.5 minutes at 85° C.; in 15 minutes at 75° C.; and in 30 minutes at 65° C. Nelson and Parmelee *(94)* noted that most phage strains survived pasteurization and some strains tolerated 70° C. for 2 hours.

Effect of chlorine. Whitehead and Hunter *(136)* state that chlorine is the most effective and convenient agent for destruction of phage. Wolf *et al. (144)* reported that fine mists containing 0.003 to 0.02% chlorine usually gave satisfactory destruction of air-borne phage. With less than 50% relative humidity, hypochlorite solutions caused slight or negligible destruction of phage. Parker

and Elliker *(103)* obtained complete destruction of phage in a preparation having a titer of 10^8 by using 50 p.p.m. sodium hypochlorite and an exposure time of 15 seconds. Bennett *(10)* inactivated air-borne phage by spraying with a hypochlorite aerosol at the rate of 0.61 g. available chlorine per 1,000 cu. ft. In a 1:2 dilution of whey, 400 p.p.m. chlorine destroyed phage in 15 seconds at pH 5.0, and in 5 minutes at pH 6.5. Chloramine-T was less effective than hypochlorite when used at the same concentration of available chlorine.

Effect of quaternary ammonium compounds. Prouty *(109)* determined the rate of phage destruction by six quaternary ammonium compounds. Although some variations were obtained, all of the compounds destroyed phage. Alkyldimethylbenzylammonium chloride was the only compound that did not inactivate phage in all trials in a concentration of 100 p.p.m. with exposures of 2 and 4 minutes. However, phage inactivation by this compound at lower concentrations was greater than with some of the other quaternaries. Exposure of cheese plant equipment to 200 p.p.m. of quaternary for 2 minutes was considered adequate for destruction of phage. Parker and Elliker *(103)* state that the most active quaternary tested by them required 50 p.p.m. and an exposure of 2 to 5 minutes for inactivation of phage in a preparation having a titer of 10^8; less active compounds required 7.5 to 10 minutes when used at the same concentration. Concentrations of 100 p.p.m. destroyed phage in 15 seconds to 5 minutes depending on the compound used. All quaternaries showed increased activity with an increase in pH. All of the quaternaries tested destroyed phage in 15 seconds with a concentration of 200 p.p.m. Bennett *(10)* noted that aerosols of quaternary ammonium compounds had an irritating effect on the respiratory tract in concentrations lower than those effective against phage. Phage in a 1:2 dilution of whey was destroyed by 1,000 p.p.m. alkyldimethylbenzylammonium chloride in 30 seconds at pH 6.6 but not at pH 4.6. Four other quaternaries gave similar results.

Effect of radiations. When bacteria-free filtrates containing phage were placed 3 in. from an ultraviolet lamp, Sutton *(120)* obtained complete destruction in 6 minutes. Phage in milk cultures treated similarly was not affected by irradiation for 1 hour. The use of irradiation hoods for decreasing the incidence of phage in cultures was suggested. Whitehead and Hunter *(138)* stated that phage active against lactic streptococci could be destroyed by irradiation if given sufficient exposure. However, the practical value of ultraviolet light for destruction of phage in a cheese plant was considered questionable because of constant reinfection. Greene and Babel *(43)* found that the rate of destruction of phage by ultraviolet light depended upon the distance of the light from the phage, the titer of the material being irradiated, the energy output of the ultraviolet lamp, the thickness of the film being irradiated, and whether phage was suspended in a liquid or irradiated in a dry state. The comparatively long time required to destroy phage at short distances from an ultraviolet lamp, and also for destruction of dry phage, appeared to limit the use of ultraviolet lamps for destroying phage in cheese plants.

Miscellanea. The inhibitory principle studied by Nelson *et al. (93)* was not affected by freezing and, at the proper pH, only slightly by drying. Crystal

violet, methylene blue, hydrogen peroxide, and potassium permanganate inactivated the principle. Hunter and Whitehead *(63)* give the following exposures for destruction of phage by various chemicals: hypochlorite (0.05%) 1 minute, potassium permanganate (0.05%) 1 to 5 minutes, hydrogen peroxide (0.5%) 1 to 24 hours, formaldehyde (0.05%) 1 to 24 hours, mercuric chloride (0.05%) 14 days, alcohol (80%) 2 to 3 days, and phenol (2.5%) 14 days. Prouty *(110)* studied the influence of pH on phage to determine whether the acid and alkaline detergents used in the dairy industry would have an effect on it. Three types of phage contained in whey filtrates showed some variation in their resistance to high pH levels; the critical levels for a 1-minute exposure varied from pH 11.3 to 11.8. At low pH levels there was less difference in the resistance of the three types, the critical levels for 1-minute exposure varying from pH 2.54 to 2.59. Because of the tolerance of phage to both high and low pH levels, it was questionable whether the ordinary alkaline and acid detergents would be of value in controlling an outbreak of phage, particularly if used at low temperatures. Bennett *(10)* found glycols to be ineffective against phage.

SOURCES OF BACTERIOPHAGE

Bacteriophage, active against lactic streptococci, was isolated by Whitehead and Cox *(134)* from an aerated culture which failed to develop acid. After propagation for several weeks, the phage preparation was of such strength that it could be diluted with several hundred billion parts of milk and still lyse a susceptible organism. Phage types were found in the atmospheres of commercial cheese factories by Whitehead and Hunter *(137)*. Finely divided particles of whey emitted from whey separators appeared to be the primary source of air-borne phage. In some cases the concentration of air-borne phage was so great that with normal technique it was impossible to prevent infection of cultures for more than a few transfers. Protection of cultures from air-borne phage eliminated failures. Hunter *(62)* stated that the only sure way of isolating a phage active against lactic streptococci was to expose the culture to air in a commercial cheese factory.

Miklik *(90)* fed material inoculated with a large number of strains of lactic streptococci to a fully grown hen and isolated from the feces a phage which was active against some of the strains. Nichols and Hoyle *(95)* isolated phage active against lactic streptococci from bulk milk, whey, cheese, and on one occasion from pig feces. Pig feces was not considered the usual source of phage.

From a study of the sources of phage in butter plants, Novák *(98)* concluded that the only regular source was cream inoculated with butter culture. The presence of phage in milk, air, and on dairy equipment was considered a secondary source. Milk drawn aseptically and the water supplies on farms did not contain phage. Anderson and Meanwell *(1)* found that a minute infection of bulk culture milk with phage will increase a thousandfold during overnight incubation in the presence of susceptible culture and also that phage will retain its full strength on storage.

Hunter *(58)* noted that the presence in cheese milk of phage active against

the lactic acid organisms used to inoculate the milk leads to its multiplication during the cheesemaking process. The phage titer of cheese whey depended largely on the initial infection. A heavy infection generally resulted in lysis of the organisms and consequent cessation of acid development. A slight infection might have no noticeable effect, and an intermediate infection generally affected the rate of acid development in the later stages of the process.

CONTROL OF SLOW ACID DEVELOPMENT DUE TO BACTERIOPHAGE

During an outbreak of slow acid production in cheese manufacture, Babel *(5)* attempted to control the slowness by the use of cultures that were not sensitive to the phage types responsible. Five selected cultures developed sensitivity to phage after use in cheese manufacture for 1 or 2 days. A culture isolated from sour cream produced acid rapidly in cheese manufacture for 3 months before it developed sensitivity towards phage, and another culture isolated from sour cream produced acid satisfactorily for more than 6 months; at the end of this time it was not sensitive to phage. Whitehead and Hunter *(139)* also report that when freshly isolated strains of *S. cremoris* or *S. lactis* were used in cheese manufacture there commonly was complete absence of the specific phage in the factory for weeks or even months. They suggested a culture rotation system for controlling phage in cheese plants in which unrelated strains of lactic streptococci would be used. Each culture in the rotation would be used every 4 days. With this procedure there would be considerable dilution of phage within the plant because of several clean-ups and applications of bactericide before the culture was used again. Nichols and Hoyle *(95)* classified 375 strains of lactic streptococci into 11 types based on their reaction to 75 phage types. Certain strains of lactic streptococci carried phage active against other strains, and it was suggested that this fact be taken into consideration when selecting cultures for rotation. Selection of cultures on the basis of only phage sensitivity was considered insufficient.

Nichols and Ineson *(96)* noted that cultures which fail to produce acid in the cheese vat may not do so after subculturing for a few days. This action was explained on the basis that commercial cultures generally contain several strains of lactic streptococci having different growth rates or responses to temperature of incubation, and one of the strains becomes prominent. If the prominent strain is attacked by phage, one of the other unaffected strains may then become prominent. Then the culture appears to have recovered. Collins *(20)* added to sterile milk a quantity of phage sufficient to give 2,500 particles per milliliter and equal amounts of a susceptible and a resistant strain of lactic streptococci. Acid production by the culture was decreased because of mass lysis of the susceptible strain, but it was greater than that of an equal portion of the resistant culture grown separately. The effect of phage on acid production was slight when susceptible bacteria were used as 50% of the total inoculum; acid production was appreciable by mixtures containing only 5 or 10% of nonsusceptible organisms. The use of equal amounts of two unrelated cultures was considered to give definite protection against culture difficulties due to phage.

Prouty *(111)* attempted to attenuate phage so that it might be used to immunize cultures. Phage-infected milk heated to 70° to 75° C. and held for 30 to 60 minutes, phage-infected whey adjusted to pH 2.5 to 11.8 and held for 1 to 30 minutes, phage-infected milk stored at 37° to 45° C., and phage-infected whey stored in the desiccated state, failed to confer any resistance on lactic acid streptococci. In the early experiments with phage, resistant cultures were visualized. However, it became apparent that cultures made resistant to a given phage soon were attacked by other phages *(65)*.

When phage acts on a sensitive culture, generally not all of the bacterial cells are destroyed; the remaining organisms are called secondary growth. The secondary-growth organisms are resistant to the phage causing lysis of the parent culture *(36)*. A culture studied by Wagenaar and Prouty *(129)* did not develop phage-resistant strains when the organisms were growing actively in milk with a very low phage titer. A phage concentration sufficient to produce mass lysis was necessary for development of phage-resistant strains. Two secondary-growth cultures were resistant to phage after holding at 0° C. for 1 month, but only one was resistant after holding at this temperature for 4.5 months.

NASCENT BACTERIOPHAGE PHENOMENON

A phenomenon by which certain cultures of lactic acid streptococci normally resistant to phage appear to be inhibited or lysed by a phage when a sensitive strain of culture is introduced was described by Evans *(34)*. Later, retarded acid development by a normally resistant mixed-strain culture, with addition of a single-strain culture and its homologous bacteriophage, was reported by Anderson and Meanwell *(1)*. Nichols and Wolf *(97)* tested 14 strains of lactic streptococci and their corresponding strains of phage for the nascent phenomenon and found four strains capable of producing the reaction. Collins *(19)* noted that inhibition of certain heterologous strains of bacteria by phage occurred in the absence of host bacteria when large numbers of phage particles were present. The homologous host, which previously was thought to be essential for occurrence of the nascent phenomenon, appeared necessary only as a means of increasing the number of phage to a point where most of the heterologous bacteria would be attacked.

Whitehead *et al. (140)* encountered instances of phage adsorption by lactic streptococci without subsequent lysis. Certain phage which exerted a lytic effect on some strains of streptococci were adsorbed by other strains without any lytic effect, and when present in relatively high concentration the adsorbed phage inhibited growth of the adsorbing organisms. With small amounts of phage present, cultures tended to recover in about 6 hours and milk usually coagulated overnight; with larger amounts of phage, coagulation of milk occurred only after 2 or 3 days. The inhibitory principle was destroyed by heat coincidentally with the phage. Therefore, inhibition was considered to be due to phage and not to some property of the *Streptococcus*. Microscopic observations by Whitehead *et al. (135)* on the nascent phenomenon showed that when the phage concentration was not too high a culture might commence growth and the organisms

increase in number for 1 to 2 hours. Later, the cocci became irregular in shape and fewer in number. Still later, growth might occur or the organisms disappear completely, depending on the proportion of phage present or on the particular strain of organism used. Dissolution, rather than inhibition, perhaps best described the phenomenon, and there was even a resemblance to lysis, although the process was longer and there was no multiplication of phage.

CONTAMINATING ORGANISMS

Morgan and Curle *(92)* noted that bacteria of the *Bacillus subtilis* type sometimes caused slow acid development in lactic cultures. However, a culture of *B. subtilis* studied by Rice *(115)* stimulated acid production by the lactic streptococci when it was growing actively in milk. The accelerated growth of the lactic acid bacteria was considered due to proteolysis by *B. subtilis*, resulting in a more available source of nitrogen than is present in fresh milk. The stimulative effect of *B. subtilis* in the cheese vat was less marked.

STREPTOCOCCI PRODUCING INHIBITORY SUBSTANCES

Rogers *(116)* reported the ability of certain lactic acid streptococci to inhibit the growth of other lactic streptococci and also *Lactobacillus bulgaricus*. Whitehead and Riddet *(141)* isolated from milk a *Streptococcus* which produced an inhibitory substance capable of delaying growth of normal lactic acid streptococci. Milk containing the inhibitory substance was referred to as "non-acid" milk. The substance was not destroyed by heating to 100° C. and holding for 30 minutes. It delayed multiplication of lactic streptococci but not the fermenting capacity of the cells *(131)*. It was readily destroyed by trypsin but was resistant to pepsin; it inhibited *S. lactis* but not *Escherichia coli* or *B. subtilis*. Streptococci which produced the inhibitory substance were detected rather frequently by Cox and Whitehead *(24)*. The organisms existed in the udders of some cows in small numbers, in cow dung, silage, and on the coats of some animals. Hunter and Whitehead *(64)* noted that milk containing inhibitory substances caused delayed coagulation much like phage. Meanwell *(89)* reported that cheese milk containing inhibitory substances was of poor bacteriological quality.

Two strains of inhibitory streptococci studied by Oxford *(102)* produced an inhibitory principle which was very active against *S. cremoris;* both strains resembled *S. cremoris*. The name diplococcin was suggested for the inhibitory substance. Mattick and Hirsch *(87)* concentrated an inhibitory substance produced by *S. lactis* which completely inhibited several organisms when used in considerable dilution. The inhibitory substance produced by *S. lactis* was not identical with that produced by *S. cremoris (49)*. The chemical behavior was dissimilar, and strains of *S. lactis* which inhibited *S. cremoris* and vice versa were isolated. The substance produced by *S. cremoris* had little or no antibacterial activity against organisms not belonging to serological group N, whereas that produced by *S. lactis* inhibited not only many streptococci but also other cocci, some aerobic and anaerobic spore-formers, mycobacteria, and some fungi; the substance was

most active against *S. cremoris*. The inhibitory substance prepared from *S. lactis* was termed nisin *(88)*. It differed from the diplococcin of Oxford *(102)* with regard to its stability in ethyl alcohol and its wide range of activity against organisms. A method for preparation of nisin was outlined, the yield being about 16 to 20 mg. per liter of culture. In the dry state, a preparation was stable for several months. Berridge *(11)* purified nisin by precipitation at pH 6.1 from phosphate buffer. By repeated fractionation, a preparation of about 30,000,000 units per gram of dry weight was prepared. Microscopic observations showed nisin to be in the form of long filaments.

Hoyle and Nichols *(56)* tested 276 strains of lactic streptococci for production of inhibitory substances; 220 of the strains were *S. cremoris* and 56 were "wild" strains. Fifty-nine (27%) of the *S. cremoris* cultures and 19 (34%) of the "wild" cultures produced inhibitory substances. The inhibitory principle produced by the "wild" strains was more active than that produced by strains of *S. cremoris*. Heating whey (pH 4.5) containing the inhibitory substance for 5 minutes at 100° C. did not decrease its activity. The antagonism between strains of *S. lactis* and *S. cremoris* was studied by Wilharm and Ciblis *(142)* using 15 strains of *S. lactis* and 8 strains of *S. cremoris* isolated from lactic cultures and 45 "wild" strains of *S. lactis* isolated from milk. With four exceptions, *S. lactis* was not inhibited by other strains of *S. lactis* or *S. cremoris* and only one strain of *S. cremoris* was inhibitory to the other seven strains. However, 40% of the strains of *S. lactis* from lactic cultures and 93% of the "wild" strains were inhibitory to *S. cremoris*. The authors suggested careful selection of strains of *S. lactis* and *S. cremoris* when combining them in new lactic cultures.

Certain strains of lactic streptococci are resistant to the inhibitory substance produced in milk by other streptococci; such cultures may or may not produce the substance. Resistant strains (classified as *S. lactis*) have been used on a commercial basis for 5 years without losing their resistance *(2)*.

The inhibitory substance of Mattick and Hirsch *(88)* inhibited the streptococci of Groups A, B, E, F, G, H, K, M, and N, as well as staphylococci, pneumococci, and several other genera. The relative sensitivity of organisms to the inhibitory substance varied; streptococci were the most sensitive. Mycobacteria were 25 to 100 times less sensitive than streptococci. The growth medium for cultivation of inhibitory streptococci influences the production of inhibitory principle *(50)*. Hirsch *(51)* reported that a single strain of *S. lactis* is capable of producing several antibiotics.

CULTURE ACTIVITY TESTS

Several tests have been advocated for determining the rate of acid production by cultures. One of the early tests was suggested by Whitehead and Cox *(132)*. The test simulated to some extent the cheese manufacturing process and was carried out in pint jars. Johns and Berard *(71)* inoculated sterile skimmilk with 1% culture and incubated it at 30° C. for 2 hours and then at 38.8° C. for 4 hours. A titration for developed acid was made each hour. A similar procedure was used by Anderson and Meanwell *(1)* except that milk was incubated for

6 hours at 30° C. and/or 37° C. Horrall and Elliker *(54)* suggested the use of reconstituted skimmilk (10%) for use in culture activity tests. For the test, 10 ml. of sterile reconstituted milk was placed in a screwcap test tube, inoculated with 0.3 ml. culture, and incubated at 37.8° C. for 3.5 hours. After incubation, the milk was titrated. Golding and McCorkle *(40)* also used reconstituted skimmilk for activity tests. The milk (100 g.) and culture (1 g.) were weighed for the test since a volume measurement of culture often varied as much as 10%. Incubation was at 30° C. for 6 hours, and the acid developed was determined by titration.

Leber *(84)* suggested an activity test based on the reduction time of resazurin. Johns *(70)* criticized this test because it failed to show differences comparable to acid production. Golding *(38)* found that methylene blue or resazurin reduction was not suitable for measuring culture activity because considerable reduction occurred in milk containing growth inhibitors.

Since increases in titratable acidity or changes in pH produced by cultures in 2 to 2.5 hours were too small to measure accurately unless a large inoculation was used, Golding *(38)* suggested an activity test based on the rate of coagulation of milk by rennet. The sample of inoculated milk was incubated at 30° C. for 2.5 hours, and the rate of coagulation of the milk was determined by a special apparatus.

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ELECTROCHEMICAL BEHAVIOR OF ION EXCHANGE RESIN MEMBRANE ELECTRODES[1]

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Membrane electrodes have been studied for some time, and an adequate theory for permeable membranes has been developed by Meyer and Sievers *(4)* and Teorell *(7)*. The theory has been recently improved and enlarged by Scatchard *(6)* to include water transfer through the membrane electrode. Marshall *(2, 3, 5)* has described membranes prepared from different clays and has modified the Teorell-Meyer-Sievers theory to bring it into accord with membranes which are not permeable to water. The heats of adsorption of ions upon the membranes has also been introduced by Marshall in order to explain the experimental membrane potentials which are obtained with simple mixtures of ions. Wyllie and Patnode *(8)* have described the preparation and electrochemical behavior of membranes prepared from various cation exchange materials using polystyrene and methyl methacrylate as bonding agents.

The study reported here describes the preparation of membranes from a number of different cation and anion exchange materials and their electrochemical behavior. Resins were selected which contained different functional groups. Amberlite IR-120, Dowex 50, Permutit Q, Amberlite IRC-50, Permutit H, Duolite CS-100, and Decalso represented the cation exchangers, and Amberlite IRA-400 and Dowex-2 were the anion exchangers chosen.

EXPERIMENTAL PROCEDURE

Preparation of the membranes. The membranes were prepared by molding an intimate mixture of the finely ground ion exchange resin and methyl methacrylate in a metallographic specimen mounting press. In each case the desired ion exchange resin was ground to pass a 200-mesh screen, and the methyl methacrylate was ground to pass an 80-mesh screen. The two materials were then

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ground together in a power mortar in the proportions, 70 ± 5% bonding material to 30 ± 5% of the air-dried exchange resin. These proportions give a membrane of maximum ion exchange resin content which retains good mechanical stability. The mixture was then molded into a membrane approximately 2.5 cm. in diameter and 1 mm. thick. A maximum temperature of 150° C. and a pressure of 4,000 lb. per square inch was used. A molding temperature of 100 to 150° C. was required to produce the necessary plasticity. At temperatures much above 150° C. structural alterations occurred as a result of the breakdown of the exchange resin. In preparing satisfactory membranes a sufficient temperature must be applied to melt the bonding plastic and enough pressure to form the disk. The actual per cent of ion exchange resin, within the limits given, did not affect the final electrochemical behavior of the membrane in any discernible manner. If much more than 30% of resin is used, the membranes tend to crumble upon prolonged soaking and lack mechanical stability, and if smaller amounts of resin are used, the resistance becomes too great for satisfactory potential measurements without electronic amplification. Membranes containing 70% bonding plastic were the most satisfactory. It was found that membranes prepared with plastic bakelite were less stable mechanically than those prepared with methyl methacrylate.

The large membranes were cut into smaller ones slightly more than 1 cm. in diameter and sealed with Cenco Sealstix or methyl methacrylate onto glass tubes 1 cm. in diameter and 4 cm. long. These glass tubes had one end flattened and ground to give a plane surface on which the membranes were cemented. The cross section of the tube was maintained at 1 cm. When the membrane is small, the possibility of leakage and the amounts of conditioning solutions needed are considerably less. There was no relationship between membrane thickness, diameter, and electrochemical response within the limits investigated (0.61 to 1.40 mm. in thickness and 1 to 2.5 cm. in diameter). However, the thicker membranes took a somewhat longer time to reach equilibrium.

The resistance of a given membrane is a function of the amount of conducting material it contains. Since the bonding plastic material is by itself an electrical insulator, the conducting substance is the exchange resin. The resistance of a given membrane will then be determined by the amount of the exchange resin material contained in the membrane. The resistances were usually about 3,000 ohms with a membrane thickness of about 0.75 mm., and individual membranes were selected so as to have approximately the same resistance values. As the membrane thickness approached 1 mm. the resistance values increased to about 5,000 to 6,000 ohms. The resistance values also serve as a measure of structural alteration. Slight variations in resistance between membranes were not considered significant, but variations of several thousand ohms were considered important and indicative of structural alterations. Amberlite IRC-50, a carboxylic type of cation exchange resin, showed a great deal of variation in the resistances of individual membranes. This variation was probably due to the electrostatic charge on the resin particles, which caused the exchange resin to agglomerate in one portion of the disk during the molding process. Permutit-H membranes also showed considerable variation in resistance values. The electrical

resistances values for Amberlite IRA-400 and Dowex-2 were uniform (3,000-5,000 ohms). Dowex-50 and Amberlite IR-120 membranes were uniform in resistance and very stable mechanically.

Potential measurements. The glass mounting tubes holding the membranes were placed in rubber stoppers which fitted 50-ml. beakers. The inside of the tube served as one side of the concentration cell, and the beaker, the other. Special calomel electrodes, of the upright type, were prepared to fit into the glass mounting tubes for use in measuring the membrane potentials. The calomel electrodes were matched to give less than 0.5 mv. asymmetry potential when both were immersed in the same solution; in most cases the asymmetry potential of the calomel electrode pair was zero.

The membranes were allowed to soak in a series of solutions of potassium chloride or other appropriate salt, of decreasing concentrations, until any initial asymmetry potential had disappeared; after this they were characterized. The characterization was accomplished by placing a solution of known activity inside the tube to which the membrane was sealed and immersing the membrane in another solution of known activity in the 50-ml. beaker. The ratio of activities inside and outside of the membrane was kept constant at one to three. This procedure gives a constant theoretical potential of 28.2 mv. for monovalent ions and 14.1 for divalent, as calculated from the Nernst equation.

The membranes used in determining the activity of potassium and calcium chloride solutions were also characterized by keeping the activity of the internal reference solution constant and increasing the activity of the external solution (Figure 2). The solutions were prepared by weighing the calculated amount of the dried salt and dissolving it in weighed quantities of double distilled water. The water was weighed on a large balance and kept to an accuracy of less than one part in 10,000. The activity coefficients used in preparing the solutions were obtained from Latimer and are the mean activity coefficients for the particular salts *(1)*. The measurements were made at room temperature (average 22° C.) with a Type K-2 Leeds and Northrup potentiometer. For each measurement the solutions were changed, both inside and outside, until a stable and reproducible potential was obtained. The selected membranes which were completely characterized were required to reproduce themselves within 0.5 mv. in solutions at activities where the membranes should obey the Nernst equation. These activities are approximately from 0.001 to 0.1. The membranes were also required to give a potential which did not deviate from the theoretical more than 1 mv., or 1.5 mv. with the calcium form membranes. If the membrane did not meet these requirements it was discarded.

The EMF data given in the tables are the actual measured potentials, as it has been found that any asymmetry potential, of chosen membranes, will disappear after one or two changes of solutions throughout the concentrations used. If any asymmetry potential is present in the membranes when the two concentrations differ, a correction may be applied by means of the characterization curve of the particular membrane in question. The activities of the characterizing solutions and the measured potentials are given in Tables 1, 2, 3, and 4.

DISCUSSION AND SUMMARY

Tables 1 and 2 contain preliminary data and show the electrochemical behavior of three types of cation exchange and two anion exchange membranes in solutions of different mono- and divalent cations. These data were instrumental in the choice of Amberlite IR-120 as the cation resin to be used in later studies. Each individual potential value in Table 1 for Amberlite IR-120 and Dowex-50 membranes represents the average of three readings for eight representative membranes—a total of 24 measurements for each activity ratio. Four different membranes were used to obtain the average potential values for Amberlite IRC-50 membranes (12 readings). A single membrane was always used to measure the potential for all activity ratios of all the respective salts; the average potential

TABLE 1

Electrochemical behavior of cation exchange resin membrane electrodes in different salt solutions

	Amberlite IR-120		Dowex 50		Amberlite IRC-50	
	Electromotive force					
Activity ratio of	*(mv.)*	Standard deviation	*(mv.)*	Standard deviation	*(mv.)*	Standard deviation
KCl						
0.00033/0.00011			23.08	2.2	22.05	5.2
0.001/0.00033			26.71	3.1	24.00	8.5
0.003/0.001	24.54	3.1	21.84	0.7	19.33	5.2
0.009/0.003	29.60	2.9	28.43	2.5	22.22	10.5
0.027/0.009	24.31	3.8	20.34	5.4	17.20	15.1
0.081/0.027	24.58	6.3	19.50	8.1	9.73	6.5
0.243/0.081	18.18	5.3	11.52	5.6	9.98	7.3
NaCl						
0.003/0.001	28.46	0.6	26.83	3.0	25.00	6.3
0.009/0.003	28.31	0.8	24.19	5.1	26.90	1.2
0.027/0.009	28.48	2.6	23.06	5.5	26.80	1.3
0.081/0.027	26.99	2.4	18.73	9.1	22.60	2.5
0.243/0.081	26.35	2.8	16.43	7.4	17.30	3.2
LiCl						
0.003/0.001	27.96	1.3	23.95	4.1	23.52	4.5
0.009/0.003	27.13	0.9	21.60	5.2	27.30	0.6
0.027/0.009	27.11	2.7	19.56	6.7	24.06	2.7
0.081/0.027	24.96	3.9	16.05	7.2	18.73	3.0
0.243/0.081	19.99	3.6	11.63	6.6	13.17	1.7
Theoretical Nernst Potential is 28.2 mv.						
$MgCl_2$						
0.0003/0.0001	14.50	1.6	14.53	5.1	13.43	2.2
0.0009/0.0003	14.50	0.6	12.67	2.5	13.93	0.4
0.0027/0.0009	12.38	3.6	9.53	4.0	12.00	2.5
0.0081/0.0027	9.58	4.6	4.31	4.6	8.57	1.7
0.0243/0.0081	7.23	3.8	−2.24	5.7	0.46	4.0
$CaCl_2$						
0.0003/0.0001	16.70	4.5	20.11	8.2	15.93	2.0
0.0009/0.0003	13.07	1.0	10.99	3.8	11.20	1.8
0.0027/0.0009	13.85	2.9	9.75	4.9	10.93	1.9
0.0081/0.0027	12.08	2.8	3.50	5.9	3.23	4.8
0.0243/0.0081	7.36	3.0	−1.45	4.8	−1.30	7.8
Theoretical Nernst Potential is 14.1 mv.						

values were then obtained from the results of all membranes used. The data shown in Table 2 for the anion membranes, Amberlite IRA-400 and Dowex-2, were obtained similarly, and each potential value is an average of 12 separate measurements.

About 25% of the membranes which were prepared met the requirements outlined above; the remainder were discarded without complete characterization. The membranes which were discarded were usually somewhat erratic in their behavior, were very slow in attaining equilibrium, or had large asymmetry potentials. It is probable that minute cracks and holes in the membrane surface account for almost all of the defective ones, and as they are heterogeneous the process of cutting them down probably caused a large number of such cracks.

It may be seen by comparing the data in Tables 1 and 2 that the anion-sensi-

TABLE 2

Electrochemical behavior of anion exchange resin membrane electrodes in different salt solutions

	Amberlite IRA-400		Dowex 2	
	Electromotive force			
Activity ratio of	*(mv.)*	Standard deviation	*(mv.)*	Standard deviation
KCl				
0.00033/0.00011	24.73	3.1	24.18	0.9
0.001/0.00033	24.93	2.5	26.55	3.2
0.003/0.001	24.83	3.1	24.12	1.3
0.009/0.003	23.48	2.3	26.18	3.1
0.027/0.009	25.40	3.4	21.75	4.5
0.081/0.027	20.50	7.7	23.48	9.6
0.243/0.081	18.48	1.8	13.68	6.3
NaCl				
0.003/0.001	25.53	1.4	27.68	0.7
0.009/0.003	24.05	1.3	24.38	1.4
0.027/0.009	23.40	1.4	23.63	1.7
0.081/0.027	24.18	1.6	23.18	4.8
0.243/0.081	21.10	0.4	18.95	3.3
LiCl				
0.003/0.001	27.62	1.3	28.82	1.5
0.009/0.003	24.60	1.3	24.33	1.6
0.027/0.009	24.35	1.5	23.38	2.9
0.081/0.027	24.40	1.4	23.73	1.8
0.243/0.081	19.15	1.4	17.55	2.8
$MgCl_2$				
0.0003/0.0001	26.30	2.6	26.32	3.9
0.0009/0.0003	28.88	1.5	28.53	1.1
0.0027/0.0009	28.83	0.8	29.93	2.1
0.0081/0.0027	30.15	2.5	28.28	0.8
0.0243/0.0081	25.83	2.4	26.83	3.6
$CaCl_2$				
0.0003/0.0001	25.68	1.1	28.68	4.0
0.0009/0.0003	27.35	2.1	28.95	1.5
0.0027/0.0009	30.13	0.6	30.23	0.3
0.0081/0.0027	27.50	0.7	28.60	2.3
0.0243/0.0081	28.15	2.5	27.83	2.0

The theoretical Nernst potential is –28.2 mv.
All of the above values are negative.

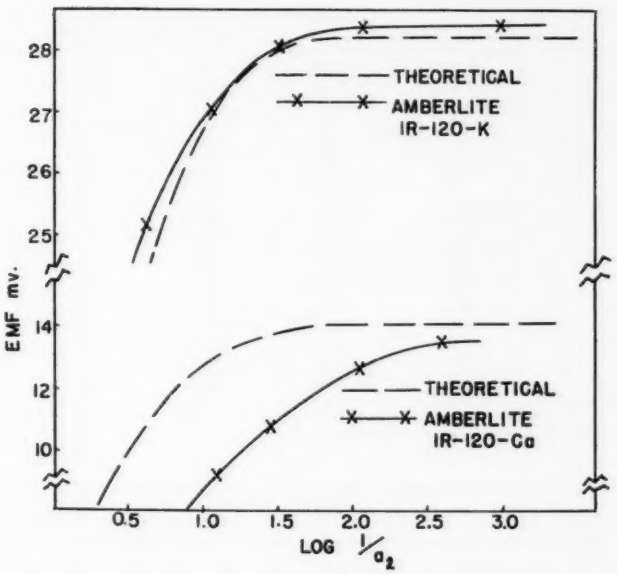

FIG. 1. Ion exchange resin membrane electrode agreement with the Teorell-Meyer-Sievers theory.

tive membranes were more reproducible and accurate than the cation membranes. Of the cation-sensitive membranes Amberlite IRC-50 membranes were the poorest and Amberlite IR-120 the best in both reproducibility and accuracy. The data in Tables 1 and 2 are to be considered as representative only of the results of a large number of unselected membranes, which were not characterized as rigorously as those selected membranes represented by the data given in Tables 3 and 4.

Tables 3 gives the characterization data of twelve selected Amberlite IR-120-K membranes. The theoretical potentials were calculated from the simpler Nernst equation for concentration cells. The data of Table 3 show the extent of obedience of the membranes to the Nernst theory of membrane potentials. In the solutions of highest activity the membrane response dropped only about an average of 3 mv. from the theoretical. The experimental potentials from each pair of activities of the solutions in the ratio, one to three, were used and the highest of the activity values were plotted in Figure 1. As is seen from Figure 1, the Amberlite IR-120-K membranes obey the Teorell-Meyer-Sievers theory very well in the concentration range studied. The method of Marshall was used to calculate the theoretical curves *(3, 5)*.

Table 4 gives the characterization data for Amberlite IR-120-Ca membranes. The averaged potentials for each activity ratio for the calcium form membranes are given in Figure 1. The calcium form membranes do not follow the Teorell-Meyer-Sievers theoretical curve as closely as the potassium form membranes;

TABLE 3
Characterization data for Amberlite IR-120-K cation membranes

Activity ratio of KCl	0.003/0.001	0.009/0.003	0.027/0.009	0.081/0.027	0.243/0.081
Membrane No.	Electromotive force in millivolts				
1	28.7	28.2	28.5	27.6	25.2
2	28.2	27.9	28.5	26.9	23.6
3	28.3	27.8	27.5	26.2	23.9
4	29.0	28.4	28.0	25.9	22.6
5	28.1	28.5	28.4	27.8	26.4
6	28.0	28.6	28.6	27.8	26.9
7	28.7	27.8	27.7	24.3	20.3
8	28.3	28.3	28.4	28.1	27.2
9	28.8	28.1	28.1	28.1	26.6
10	28.6	28.7	28.5	27.8	26.2
11	28.6	28.4	27.9	27.6	25.8
12	28.4	28.5	28.3	27.5	25.4
Average	28.5	28.3	28.2	27.1	25.0
Standard deviation	0.32	0.32	0.39	1.14	2.05

The theoretical Nernst potential is 28.2 mv.

however, they obey the Nernst equation for concentration cells fairly well, as may be seen from Figure 2. The agreement of the Amberlite IR-120-K membrane potentials with the theoretical calculated from the Nernst equation for concentration cells is also shown in Figure 2. The average potentials and calculated standard deviations for selected Amberlite IR-120-K and Ca membranes are given for each activity ratio. The potassium form membranes gave more reproducible potentials and agreed more closely with the theoretical values than did the Ca membranes.

The results of the study show that when the ion exchange resin membrane electrodes have been completely characterized they may be used to determine the activities of monovalent or divalent salts when the same salt is used as the reference. In solutions of activities from 0.001 to 0.1 the Nernst equation holds fairly well, but in solutions containing a greater activity than approximately

TABLE 4
Characterization data for Amberlite IR-120-Ca cation membranes

Activity ratio of $CaCl_2$	0.003/0.001	0.009/0.003	0.027/0.009	0.081/0.027	0.243/0.081
Membrane No.	Electromotive force in millivolts				
1	13.6	12.8	10.4	9.5	7.4
2	13.1	13.3	10.0	6.8	4.5
3	13.9	12.6	11.0	9.8	7.4
4	13.1	13.4	11.4	12.8	5.0
5	13.6	12.7	10.8	9.1	7.0
6	13.4	12.1	9.8	7.8	5.3
7	12.7	13.1	10.6	10.2	8.3
8	13.5	12.8	10.8	9.6	7.3
9	13.9	12.7	10.3	8.4	6.3
10	12.4	14.3	6.8	9.6	6.7
Average	13.3	13.0	10.2	9.4	6.5
Standard deviation	0.52	0.60	1.28	1.59	1.25

The theoretical Nernst potential is 14.1 mv.

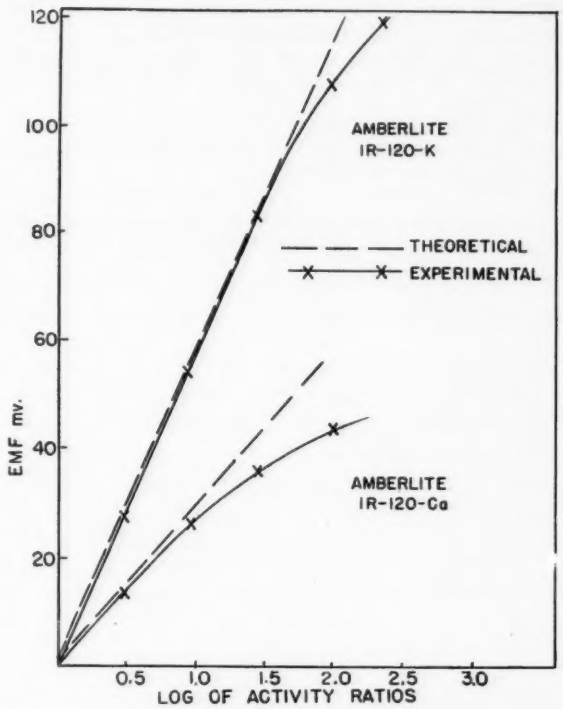

FIG. 2. Ion exchange resin membrane electrode agreement with the Nernst equation for concentration cells. Activity of the reference solution of KCl was 0.001.

0.1 recourse must be made to the characterization curve of the particular membrane electrode in question in order to determine the activity of a particular salt solution.

These membrane electrodes have been used in studies on the activity of the cations and anions in milk and the interaction of the salts common in milk with casein sols, the results of which will be published in subsequent papers.

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THE INFLUENCE OF CERTAIN FACTORS ON THE BACTERIAL COUNTS AND PROTEOLYTIC ACTIVITIES OF SEVERAL PSYCHROPHILIC ORGANISMS[1]

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Although the importance of psychrophilic bacteria in dairy products was recognized more than 50 years ago *(2, 7)*, this problem recently has received considerable attention because of adoption of processing and marketing procedures which tend to increase the time between processing and consumption of milk.

The literature reveals many studies on different aspects of this problem; however, few have been conducted on the growth characteristics of dairy psychrophiles in pure culture. Recently, Greene and Jezeski *(10)* and Lawton and Nelson *(12)* reported on the growth characteristics and biochemical activities of several psychrophilic bacteria in pure culture.

The present study with pure cultures of psychrophilic bacteria was undertaken to investigate *(a)* the influence of time and temperature of plate incubation on bacterial counts, *(b)* the influence of time and temperature of storage on the growth characteristics, and *(c)* the proteolytic activities at different holding temperatures.

EXPERIMENTAL METHODS

The cultures of psychrophilic bacteria used were from a group of pure cultures isolated in a previous study *(14)*. The cultures were identified by reference to Breed *et al. (4)*. Cultures 1 and 3 belonged to the genus *Pseudomonas* but did not fit the characteristics of any of the species described by Breed *et al.* Culture 2 was tentatively identified as *Pseudomonas fluorescens,* culture 4 as *Ps. putrefaciens.*

The stock cultures were carried in sterile litmus milk at 5° C. and were transferred every 10 days. Milk for the trials consisted of fresh skimmilk which was autoclaved for 15 minutes at 15 lb. steam pressure. In experiments involving storage of samples at different temperatures, inoculated skimmilk was mixed thoroughly and transferred aseptically to sterile screw-cap bottles to provide samples for each storage temperature. In each case 0.1% of a culture grown at 5° C. for 10 days was used as inoculum.

In plating, tryptone–glucose–extract agar *(9)* was used. Time and temperature of storage and plate incubation will be given for each trial separately in the section on results. Duplicate plates of each dilution were made for each time and temperature combination.

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Proteolysis was measured by the production of soluble nitrogen, and tyrosine and tryptophan. Nitrogen fractions soluble in trichloroacetic acid were determined by a Kjeldahl procedure *(15)*. Tyrosine and tryptophan were determined colorimetrically in a trichloroacetic acid filtrate as described previously *(15)*. All chemical determinations were made in duplicate.

RESULTS

Influence of plate incubation time and temperature on bacterial counts. Cultures 1, 2, 3, and 4 were inoculated individually into sterile skimmilk and held at 25°, 21°, 10°, and 5° C. for 16 days. Samples of these cultures were plated daily and incubated at 25° C. for 2 and 3 days, at 21° C. for 2, 3, and 4 days, and at 10° and 5° C. for 5, 7, and 10 days.

TABLE 1

Effect of plate incubation time on counts of culture 1 at different plate incubation temperatures[a]

Incubation temperature	Per cent of count after maximum plate incubation time						
	25° C.	21° C.		10° C.		5° C.	
Incubation time	2 of 3 d.[b]	2 of 4 d.	3 of 4 d.	5 of 10 d.	7 of 10 d.	5 of 10 d.	7 of 10 d.
Mean	96	85	96	82	89	51	70
Median	97	86	97	77	91	46	73
Range	81-111	78-97	82-103	62-101	82-109	24-77	40-105

[a] Culture 1 was held at 5° C.
[b] Count at the shorter incubation time (2 days) expressed as per cent of the count obtained after maximum plate incubation time (3 days).

Data on the effect of plate incubation time on the counts of culture 1 (held at 5° C.) at different plate incubation temperatures are presented in Table 1. For each plate incubation temperature the total count of each sample at the shorter plate incubation time was expressed as per cent of the count obtained after maximum plate incubation time. The means then were calculated arithmetically from the data obtained from daily platings. The counts at the shorter incubation periods usually were somewhat lower than at maximum plate incubation time, and the colonies frequently were more difficult to count because of the small size. This was more frequently observed at the lower plate incubation temperatures. The results at the other holding temperatures and those on cultures 2, 3, and 4 were very similar to those obtained with culture 1 held at 5° C.

Figure 1 shows the influence of plate incubation temperature on the counts of cultures 1 and 2 after holding at 5° C. Plates were incubated at 35° and 32° C. for 2 days, at 25° C. for 3 days, at 21° C. for 4 days, and at 10° and 5° C. for 10 days. Incubation at 32° and 25° C. gave maximum counts for culture 1. Somewhat lower counts were found at 21°, 10°, and 5° C. The counts at 35° C., however, were much lower than at the other incubation temperatures. In culture 2, the counts at 25°, 21°, 10°, and 5° C. were very close. An incubation temperature of 25° C. apparently was the upper limit for culture 2; 32° C. for culture 1. Although the shape of the growth curves at holding temperatures of 25°, 21°,

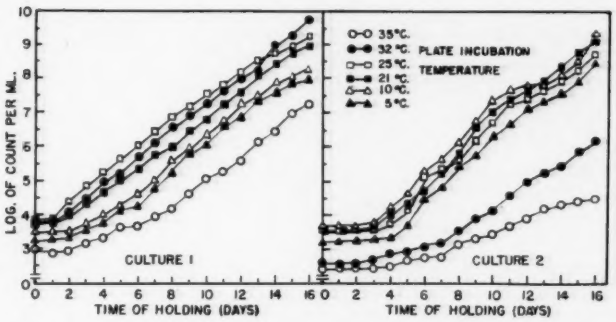

FIG. 1. Influence of plate incubation temperature on the counts of cultures 1 and 2 when held at 5° C.

and 10° C. differed from that at 5° C., the effect of incubation temperature on the counts at the other holding temperatures was essentially the same as at 5° C. The results with cultures 3 and 4 were similar to those of culture 1.

Influence of holding temperature on bacterial counts. The pure cultures were inoculated separately into sterile skimmilk and stored at 5°, 10°, 21°, 25°, 32°, and 35° C. for 16 days. Samples of these cultures were plated daily with incubation at 25° C. for 3 days. Figure 2 shows the influence of storage temperature on the counts of cultures 1 and 2. For both cultures the growth curves at 25° and 21° C. were very similar. The population at 5° and 10° C. (culture 1) rose to the same level but at a slower rate. At 5° C. an increased lag phase was observed. The maximum population at 35° and 32° C. was surpassed by those at the other temperatures. Culture 2 did not grow at 35° nor at 32° C. and showed a lag phase of at least 48 hours at all temperatures. After 7 days the population at 10° C. surpassed those at 25° and 21° C. and continued to increase to a much higher level. The same was true after 11 days for the sample held at 5° C. The results with cultures 3 and 4 were very similar to those obtained with culture 1.

Generation time and maximum population. The generation time was calculated from the growth curves for each sampling interval at each holding temperature (see Figure 2). Minimum generation time and maximum population and the sampling interval from which these data were obtained are presented in Table 2. In cultures 1 and 3 the rate of cell division at 5° and 10° C. was slower than at the other temperatures. The maximum viable populations at the different temperatures were very close except at 35° C.; however, at the lower temperatures increases in count were found even after 15 days. Minimum generation time in culture 1 was obtained within 2 days at all storage temperatures; in culture 3, within 3 days. The data also show that culture 2 had its optimum temperature for increase in population at 10° and 5° C. The results on culture 4 were very similar to those of culture 1.

Proteolytic activities. During the trial on the influence of storage temperature on growth (Figure 2), samples were taken at regular intervals for soluble nitrogen and tyrosine and tryptophan determinations. Figure 3 shows the effect

TABLE 2

A comparison of minimum generation times and maximum counts at various holding temperatures

No. of culture	Holding temperature of samples					
	5° C.	10° C.	21° C.	25° C.	32° C.	35° C.
1	1-2[a]	1-2	0-1	0-1	0-1	0-1
	864[b]	618	132	130	130	150
	2×10^{9}[c]	3.2×10^{9}	2.5×10^{9}	1.3×10^{9}	1.3×10^{9}	7.9×10^{7}
	16[d]	16	6	3	3	2
2	5-6	5-6	4-5	4-5		
	432	360	480	546		
	6.3×10^{8}	2.5×10^{8}	1.3×10^{7}	5×10^{6}	no growth	no growth
	16	16	9	9		
3	2-3	2-3	0-1	0-1	0-1	0-1
	721	570	120	117	125	155
	1.3×10^{9}	2.3×10^{9}	3.2×10^{9}	5×10^{9}	5.2×10^{8}	1.3×10^{7}
	16	16	5	4	3	2

[a] Interval (days) in which the minimum generation time was obtained.
[b] Minimum generation time (min.).
[c] Maximum viable count per ml.
[d] Day at which maximum count was obtained.

of holding time and temperature on the production of soluble nitrogen and tyrosine and tryptophan in culture 1.

At 25° and 21° C. increases in soluble nitrogen and tyrosine and tryptophan were noticeable within 24 hours when the cell count was about 10^7 cells per milliliter. At 10° and 5° C., however, proteolysis was negligible during the first 5 days but became detectable when the population reached 10^6 to 10^7 cells per milliliter and continued to increase throughout the test period. The production of soluble nitrogen and tyrosine and tryptophan at 25° and 21° C. was rapid during the first 7 days when the cell population reached a maximum. Later, when the number of viable cells was declining, the rate of soluble nitrogen and tyrosine and tryptophan production also declined; however, there was a gradual increase in soluble nitrogen and tyrosine and tryptophan throughout the test period. The results indicate that proteolysis in culture 1 was noticeable when the population had increased to 10^6 to 10^7 cells per milliliter.

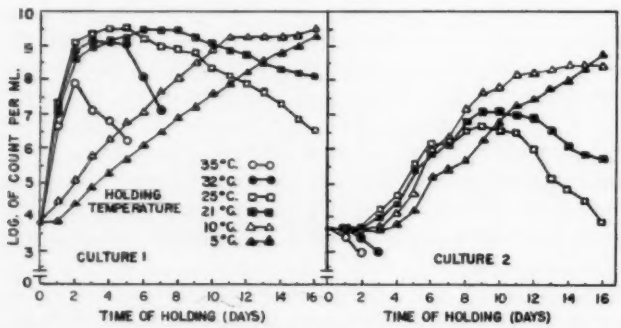

FIG. 2. Influence of holding temperature on the counts of cultures 1 and 2.

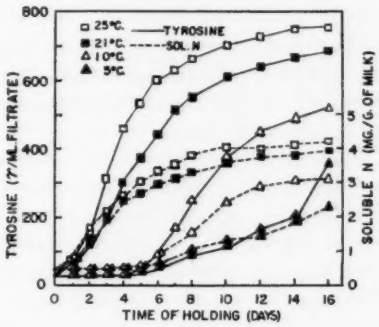

FIG. 3. Proteolytic activity of culture 1 at different holding temperatures.

Cultures 1, 2, 3, and 4 were proteolytic to varying degrees as determined by the production of soluble nitrogen and tyrosine and tryptophan. The possibility of a relationship between cell count and proteolytic activity was investigated.

Figure 4 shows the bacterial counts and proteolytic activities of cultures 2 and 3 after holding at 5° and 25° C. Although the growth curves of cultures 1 and 3 were very similar at both 25° and 5° C., marked differences were found in their proteolytic activities. At 25° C., culture 1 showed definite increases in tyrosine and tryptophan within 24 hours. Proteolysis in culture 3, however, was noticeable after 8 days when the cell count was 10^8 cells per milliliter and the number of viable cells was declining. At 5° C., proteolysis in culture 3 was detectable about 6 days later than in culture 1, although culture 3 had reached the same level of population (about 10^6 cells per milliliter) 2 days earlier than culture 1. Proteolysis in culture 2 at 25° C. was noticeable at a count of 10^5 to 10^6 cells per milliliter; at 5° C., at a count of 10^7 cells per milliliter.

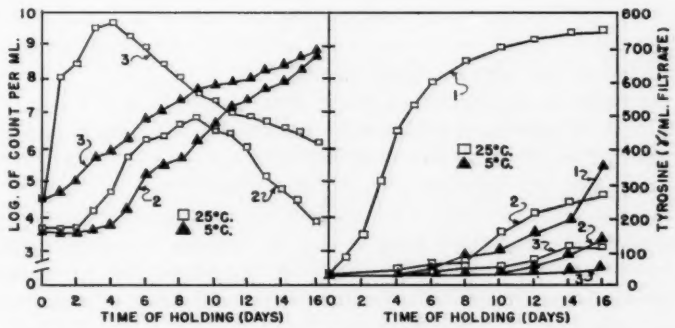

FIG. 4. A comparison of bacterial counts and proteolytic activities of cultures 2 and 3 when held at 5° and 25° C.

DISCUSSION

One of the objections to growing pure cultures of psychrophilic bacteria in sterile skimmilk could be that their growth characteristics and certain biochemical activities in mixed flora, such as in raw or pasteurized milk, may differ from those of pure cultures in sterile skimmilk. Although changes in environment are undesirable and may limit the interpretation of results somewhat, it was thought that the use of pure cultures under controlled conditions of time and temperature is to be preferred in studies of this type. Recently, Lawton and Nelson *(12)* reported that the growth curves of psychrophiles in sterile skimmilk were very similar to those obtained with laboratory-pasteurized milk.

In the classification of some of the psychrophilic bacteria according to Breed *et al. (4)*, difficulties sometimes were encountered in assigning the species name. The results of certain biochemical tests were different when different incubation temperatures were used. These variations are important not only to the taxonomist but also to the dairy industry, since certain activities, such as proteolysis, may occur at one temperature and not at all or to a lesser extent at another temperature. In this study an incubation for 3 days at 25° C. was used, since this temperature was optimum for growth of most of the psychrophiles studied.

Incubation for periods shorter than 3 days at 25° C., 4 days at 21° C., and 10 days at 10° or 5° C. gave lower counts than at maximum plate incubation time. A plate incubation for 3 days at 25° C. seemed preferable for the enumeration of the psychrophiles in this study. Incubation at 35° C. gave consistently lower counts. These results are similar to those found for refrigerated milk and ice milk *(13, 14)*.

Although an incubation of 7 days at 5° C. is recommended by *Standard Methods (1)* for the enumeration of bacteria which grow under refrigeration, the colonies at this time frequently are difficult to count because of the small size. A plate incubation for 3 days at 25° C. seems preferable since the counts are available sooner and less equipment and space are necessary.

The growth curves show that all organisms, with the exception of culture 2, were able to grow over a rather wide range of temperatures. They probably should be termed facultative psychrophiles, since they grow better at 21° C. or above. Culture 2, which grew better at 10° and 5° C. and not at all at 32° and 35° C., is a more truly psychrophilic organism.

The lag phase of all organisms, except that of culture 2, did not exceed 24 hours, which is in agreement with results reported by Kaufmann and Andrews *(11)*. However, in refrigerated pasteurized milk with a mixed flora, lag phases of 3 to 5 days have been reported *(5, 6, 8)*. It is possible that initiation of growth is more rapid in pure cultures in a somewhat different growth medium, such as sterile skimmilk. On the other hand, it may be that the heat treatment of pasteurization has some effect on the extent of the lag phase. The presence of only a small number of psychrophiles in relation to the total count in pasteurized milk may explain the long lag phase sometimes found.

Considerable variation was found in the proteolytic activities of the four cultures. This may be caused by a difference either in type, amount, or activity

of enzyme. Proteolysis at the higher temperatures was more rapid, which may be due to either increased production or activity of the enzyme at the higher temperatures. Proteolysis was noticeable at plate counts ranging from 10^5 to 10^9 cells per milliliter. No direct relation could be established between level of population and proteolytic activity.

After holding at 25°, 21°, 10°, and 5° C. for 16 days, the amount of soluble nitrogen in culture 1 expressed as per cent of total nitrogen was 78, 74, 58, and 45, respectively; the figure at the beginning of the incubation period was about 10%. When culture 1 was held at 25°, 21°, 10°, and 5° C. for 16 days the amount of free tyrosine and tryptophan expressed as per cent of all tyrosine and tryptophan present in milk *(3)* was 85, 80, 58, and 40, respectively. Although the relative closeness of these figures probably is fortuitous, a similar relationship between production of soluble nitrogen and tyrosine and tryptophan was found in a previous study with *Streptococcus lactis (15)*.

SUMMARY

A plate incubation for 3 days at 25° C. is recommended for the enumeration of bacteria growing in refrigerated milk and related products. Three of the four psychrophiles studied were able to grow over a wide range of temperatures. With the exception of one, all organisms had a short lag phase even at the lower holding temperatures. No relation was evident between level of population and proteolytic activity. There appeared to be some relationship between the values for the production of soluble nitrogen and tyrosine and tryptophan.

ACKNOWLEDGMENT

The authors are indebted to B. Tennison for his technical assistance.

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AN EXTRACTION-TITRATION METHOD FOR THE DETERMINATION OF FREE FATTY ACIDS IN RANCID MILK AND CREAM[1]

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The titration of free fatty acids in fat derived from rancid milk and cream has generally been accepted as the most reliable quantitative method for measuring the extent of lipolysis. However, the method of obtaining fat for titration affects its acid degree. Johnson and Gould *(12)* found that the solvent extraction of fat from rancid cream gives a greater recovery of fatty acids than does churning. In a study of the partition of free fatty acids among different phases upon the churning of rancid cream, Tarassuk and Frankel *(19)* established that about 75% of the added low-molecular-weight fatty acids (butyric, caproic, and caprylic acids) are lost in the buttermilk and as much as 30% of the added high-molecular-weight fatty acids (palmitic and stearic acids) are retained in the foam phase that is formed in the melting of butter. These limitations of the acid degree determination of churned fat emphasize the need for a method of determining free fatty acids directly in milk and cream.

Extraction-titration methods have been described for determining fatty acids in rancid milk *(15)* and artificial emulsions *(7, 16, 17)*, and the water-insoluble fatty acids in cream *(1)*. These procedures are based on the extraction of lipid material with fat solvents and the subsequent titration of free fatty acids in the solvent extract.

No reliable information is available concerning the recovery of free fatty acids that can be obtained by solvent extraction methods. The recovery data of Johnson and Gould *(12, 13)* and Nashif and Nelson *(16)* were based on the extraction of a limited number of fatty acids that had been previously added in solution directly to cream. The origin of fatty acids in rancid milk is in the fat phase. Upon hydrolysis of fat the partition of fatty acids between fat and water phases will depend upon the relative solubility of each acid in these phases. This partition will be further modified by the mutual solubility of the acids. Since the fatty acids (except butyric acid) are slightly soluble or insoluble in water, their distribution in the water and fat phases when added directly to cream will not be comparable to their distribution in natural rancid cream. Therefore, for the recoveries studies reported in this paper, milk fat to which known amounts of fatty acids were added was emulsified in fresh skimmilk. These fatty acids were representative of the fatty-acid composition of milk fat. It is considered that emulsions so prepared are more nearly comparable to the natural emulsion of rancid milk than milk or cream to which fatty acids have been added directly.

A simple extraction-titration method applicable directly to milk and cream is described in this paper. This method is based on the solvent extraction pro-

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[1] A preliminary report of this work has been made *(19)*.

cedure of Johnson and Gould *(12)*. The development of the procedure and evaluation of the results are based on determinations of recovery of fatty acids from emulsions prepared as described above.

EXPERIMENTAL PROCEDURE

Extraction-titration procedure. 10.0 ml. of milk is pipetted into a 50-ml. centrifuge tube and 10 ml. of 95% neutralized ethanol is added. The firmly stoppered tube is shaken vigorously for 1 minute, 15.0 ml. of a mixture of 40% ethyl ether and 60% petroleum ether[2] is added, and the tube is shaken for another minute. The tube is then centrifuged at approximately 1,500 r.p.m. for 3 minutes. An aliquot portion of 5.0 ml. is pipetted from the supernatant clear ether layer and mixed in a 125-ml. Erlenmeyer flask with 15 ml. of 95% ethanol containing five drops of 1% alcoholic phenolphthalein and previously neutralized to a pink color. This mixture is titrated with 0.0250 *N* alcoholic KOH to the same color, using a 2-ml. burette graduated to 0.010 ml. The titration reading is multiplied by three to obtain the volume of alkali necessary to neutralize the total ether layer. The results are expressed as "free fat acidity" (F.F.A.), i.e., milliliters of 1 *N* alkali required to neutralize an ether extract of milk or cream containing 100 g. of fat. In order to calculate this value, the fat is determined in the samples by the Babcock method. The "free fat acidity" is equivalent to the "acid degree" used for the titration of fat.

The same procedure is applied to cream, except for the use of a smaller sample—one containing the same amount of fat as in 10 ml. of 4% milk. The cream sample is weighed on a moisture and butterfat torsion balance (sensitivity ± 30 mg.).

Materials. Fresh, grade A raw milk from the University Creamery was used in all experiments. Lipolysis was induced by homogenizing raw milk at temperature of 35° C. and pressure of 2,000 p.s.i. The milk was subsequently cooled to 4° C. and stored at this temperature. When lipolysis became sufficient to lower surface tension to about 36 dynes per centimeter *(6, 18)*, the milk was heated to 80° C. to inactivate the lipase. Rancid cream was prepared in the same way as rancid milk. Samples of varying degrees of rancidity were obtained by mixing rancid milk or cream with different proportions of fresh pasteurized milk or cream of the same fat content.

Milk fat was obtained from cream either by solvent extraction or by churning mechanically in Dazey glass churns. The butter was melted in a 60° C. oven until three distinct layers appeared. The upper foam layer was removed and the clear fat was decanted and filtered through No. 1 Whatman fluted filter paper in the oven. The solvent extraction procedure used to obtain milk fat from cream was essentially that of Johnson and Gould *(12)*. The fats produced by both methods were stored at 0° C. until analyzed.

Recovery experiments were carried out on milk emulsions made with milk fat to which fatty acids[3] of different molecular weight were added in varying

[2] Boiling range: 35-60° C.

[3] Obtained from the Eastman Kodak Company, Rochester, New York.

amounts. The fat used for this purpose was in some cases prepared by removing the small amounts of fatty acids that are present in fresh milk fat with the method of Breazeale and Bird *(3)*. The acid degree of the "acid-free" fat obtained by this method generally varied from 0.150 to 0.200. A more effective and simple method for the removal of fatty acids from fresh milk fat was described by Borgström *(2)*, i.e., passing milk fat in wet ethyl ether through an ion exchange column of Amberlite IRA 400.[4] The fat obtained by this method had an acid degree of approximately 0.050. This method was used in one experiment, where the most complete removal of fatty acids was necessary.

The weighed fatty acids were added to batches of fat of 100 g. each which were then heated in a water bath until the fatty acids dissolved. These fats were then emulsified in fresh pasteurized skimmilk at 37° C. by passing them through the homogenizing valve of a hand emulsifier, using a positive pump, under a pressure of 250 p.s.i., to give a 4% milk. The resulting emulsions showed microscopically a fat globule size distribution comparable to that of normal non-homogenized milk.

Methods. The acid degree of churned and extracted fats was determined essentially by the method of Herrington and Krukovsky *(9)*—titrating 5.00 g. of fat with 0.050 *N* alcoholic KOH in the presence of neutralized ethanol at 60° C.

Surface tension measurements were made in triplicate with a Cenco-du Noüy surface tensiometer at 20° C.

The pH of milk was determined with a Beckman pH meter.

RESULTS AND DISCUSSION

Titration of the ether extract. The reliability of the titration of a 5-ml. aliquot of ether extract was studied by comparing it to the titration of the entire ether layer. This latter procedure was carried out by the use of a siphon similar to that described by Hillig *(10)*, and two extractions with 15 and 10 ml. of ether mixture, respectively. The results of Table 1 show that for rancid milk the titration of the entire ether layer yielded somewhat higher values than did the titration of a 5-ml. aliquot of the ether extract; for fresh milk it yielded lower blank values. However, the titration of the aliquot portion gave more uniform results than did the titration of the total ether layer. The titration of a 5-ml. aliquot portion of the ether extract was therefore chosen because of its simplicity and reproducibility in routine analyses.

A suitable indicator was chosen on the basis of a potentiometric curve established with a titration mixture from rancid milk. Figure 1 shows that phenolphthalein and the mixed indicator of Kleinzeller and Trim *(14)* provide suitable end points. Phenolphthalein was chosen because its end point was closer to the neutralization point (pH 10.3-10.4) than was that of the indicator of Kleinzeller and Trim.

Determination of the proper ratio of ethyl ether to petroleum ether. The optimum solvent ratio to use in the present extraction-titration procedure was

[4] Donated by the Rohm and Haas Company, Philadelphia, Pa.

TABLE 1

Comparison between titration of 5 ml. aliquot of ether layer and titration of total ether layer

Procedures		Homogenized milk			
		Fresh		Rancid	
		(ml. 0.025 N NaOH per 10 ml. milk)	*(% deviation from average)*	*(ml. 0.025 N NaOH per 10 ml. milk)*	*(% deviation from average)*
I. Titration of 5 ml. aliquot of ether layer	(1)	0.408	3.55	3.622	0.385
	(2)	0.378	4.06	3.645	0.248
	(3)	0.398	1.01	3.678	1.16
	(4)	0.391	0.762	3.600	0.990
	Average	0.394		3.636	
II. Titration of total ether layer	(1)	0.286	5.92	3.898	0.154
	(2)	0.345	13.5	3.968	1.95
	(3)	0.275	9.54	3.910	0.463
	(4)	0.311	2.30	3.790	2.62
	Average	0.304		3.892	

determined on the basis of recovery of fatty acids from milk emulsions. Since the ether layer obtained by the procedure under investigation varied in volume when different ratios of ethyl ether to petroleum ether were used, the procedure consisting of titrating the entire ether layers derived from two extractions described previously was used for this experiment.

The above procedure was applied to rancid and fresh milk and to artificial milk emulsions made with fresh fat and with fresh fat to which were added equal molar quantities, respectively, of low-molecular-weight fatty acids (bu-

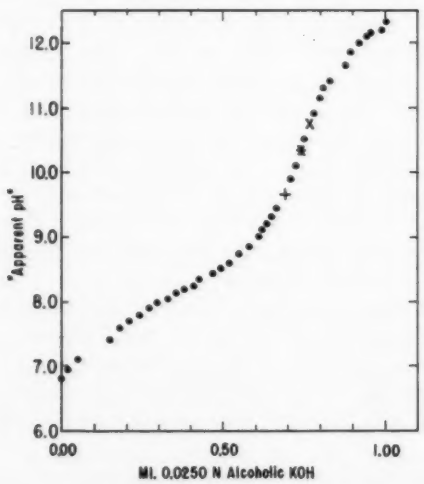

FIG. 1. Potentiometric curve of titration mixture obtained from rancid milk (× = end point of phenolphthalein, * = neutralization point, + = end point of indicator of Kleinzeller and Trim).

TABLE 2

Effect of varying the ratio of ethyl ether to petroleum ether on the extraction of fatty acids

Solvent ratio		Artificial emulsions made with:							Homogenized milk		
Ethyl ether	Petroleum ether	Fresh fat[a]	Fat + butyric, caproic and caprylic acids[a]			Fat + myristic, palmitic and stearic acids[a]			Fresh	Rancid	
(ml.)	*(ml.)*	*(F.F.A.)*	*(F.F.A.)*	*(Net F.F.A.)*[b]	*(% Recovery)*	*(F.F.A.)*	*(Net F.F.A.)*[b]	*(% Recovery)*	*(F.F.A.)*	*(F.F.A.)*	*(Net F.F.A.)*[c]
100	0	2.42	5.75	3.33	75.8	5.20	2.78	61.2	2.42	12.5	10.1
75	25	2.42	5.54	3.12	71.1	5.20	2.78	61.2	2.49	12.2	9.71
66.6	33.3	2.21	4.50	2.29	52.2	5.20	2.99	65.8	2.21	11.6	9.39
50	50	0.693	3.12	2.43	55.4	4.92	4.23	93.2	2.21	10.4	8.19
40	60	0.208	2.49	2.28	52.0	4.50	4.29	94.5	0.970	9.70	8.73
33.3	66.6	0.140	2.42	2.28	52.0	4.50	4.36	96.0	0.900	9.54	8.64
25	75	0.140	1.59	1.45	33.0	4.43	4.29	94.5	0.900	9.40	8.50
0	100	0.140	0.692	0.552	12.6	4.43	4.29	94.5	0.900	6.49	5.59

	Acid degree before emulsification
[a] Fresh fat	0.304
Fat + butyric, caproic and caprylic acids	4.39
Fat + myristic, palmitic and stearic acids	4.54

[b] Net F.F.A. = F.F.A. – F.F.A. of corresponding emulsion made with fresh fat.

[c] Net F.F.A. = F.F.A. – F.F.A. of corresponding fresh milk.

tyric, caproic, and caprylic acids) and high-molecular-weight fatty acids (myristic, palmitic, and stearic acids). Recoveries in the milk emulsions were calculated from the acid degree of the fats before emulsification and the free fat acidity values of the corresponding emulsions.

The results presented in Table 2 reveal that as the ratio of petroleum ether to ethyl ether increases, recovery of low-molecular-weight fatty acids decreases and recovery of high-molecular-weight fatty acids increases, the latter reaching a constant value when petroleum ether is 60% or more of the solvent mixture. High free fat acidity values were obtained with the emulsion made with fresh fat and with fresh homogenized milk when the proportion of ethyl ether was increased above 40%. This indicates that a portion of the aqueous phase of the milk would be extracted in the ethereal layer, thus contributing to the free fat acidity. On the basis of these results, a ratio of 40% ethyl ether and 60% petroleum ether was considered to provide the highest recovery of fatty acids with the least extraction of the aqueous nonfatty acid constituents that would contribute to the free fat acidity. This solvent ratio, based on recovery of added fatty acids, proved to be the same as that of Johnson and Gould *(12)*, which was based on acid degree values and percentage recovery of solvent extracted milk fat from cream.

Effect of the pH of rancid milk on the recovery of fatty acids. Johnson and Gould *(13)* reported that a higher recovery of fatty acids from milk or cream results from acidification to pH 2 with sulfuric acid before extraction with solvents. They stated that negative qualitative tests obained for sulfate ions indicated that no entrained sulfuric acid resulted from this procedure. Nashif and Nelson *(16)* also acidified their samples to a pink color with thymol blue before extraction with solvent.

The effect of pH on the recovery of fatty acids was studied on rancid milk and milk emulsions containing low-molecular-weight and high-molecular-weight fatty acids. A solution of sulfuric acid (25% by volume) was added to measured samples of milk in the centrifuge tubes to obtain pH values ranging from 5.0 to 1.0. Representative results from several experiments in Table 3 show an increase in free fat acidity as pH was lowered. When pH was lowered below 5.0, the

TABLE 3

Effect of lowering the pH of rancid milk and milk emulsions with sulfuric acid on the free fat acidity and recovery of fatty acids

pH	Rancid milk	Milk emulsion of fat + butyric, caproic, and caprylic acids[a]		Milk emulsion of fat + myristic, palmitic, and stearic acids[b]	
	(F.F.A.)	*(F.F.A.)*	*(% recovery)*	*(F.F.A.)*	*(% recovery)*
6.5	20.5	3.62	58.2	6.13	96.0
5.0	22.0	3.87	62.2	6.45	101
4.0	22.3	3.87	62.2	6.72	105
3.0	22.7	4.16	66.8	6.96	109
2.0	23.7	4.38	70.4	7.22	113
1.0	23.8	6.20	99.6	7.74	121

[a] Acid degree before emulsification: 6.22.
[b] Acid degree before emulsification: 6.38.

emulsion containing high-molecular-weight fatty acids showed recoveries of fatty acids that exceeded 100%. This may be the result of a number of factors, as follows: *(a)* entrained sulfuric acid in the ether layer; *(b)* shift of the phosphate equilibrium in milk from HPO_4^{--} to $H_2PO_4^-$ and H_3PO_4, the latter of which may be carried over in the ether extract; *(c)* hydrolysis of milk fat by the added acid. The first possibility could not be verified since it is difficult to test for small amounts of sulfate in the ether layer. The phosphorus contents of the fats obtained from rancid milk to which sulfuric acid was added prior to extraction and the same milk without sulfuric acid were 0.238 and 0.174 mg. per gram of fat, respectively. The recovery of fat in the ether layer was the same in both milks. This indicates that the second factor plays some role in the increased free fat acidity. This contribution of phosphoric acid to the increased free fat acidity was confirmed by the excessively high free fat acidity value obtained in fresh milk (5.50) when phosphoric acid (85%) was used to lower the pH of milk to 2 prior to solvent extraction. In order to determine whether fat hydrolysis had occurred in the rancid milk to which sulfuric acid was added (pH 2), titrations were made of both the ether and aqueous layers obtained after extraction of this milk, and of the same milk without added sulfuric acid. The difference between the total acidity of the ether plus aqueous phase in the two milks (1.0 milliequivalent) in no case exceeded the acidity of the added sulfuric acid (1.1 milliequivalent). This precludes the possibility that the acid hydrolyzed the fat.

Since the evidence indicates that the higher free fat acidity obtained in milk when its pH is lowered to 2 with sulfuric acid before solvent extraction is due partly to factors other than higher recovery of fatty acids, the adoption of this procedure in the extraction-titration method was not deemed justified. Whether there is actually a greater recovery of fatty acids as a result of lowering the pH of milk can be established only by determinations of individual fatty acids in the ether extract.

Effect of lactic acid in rancid milk on the free fat acidity. It has been reported that the presence of lactic acid in rancid cream gave a greater increase in acid degree in churned fat than in solvent-extracted fat *(12)*.

Varying amounts of lactic acid (7% by volume) were added to a series of samples of rancid milk. Determinations were made of free fat acidity by the extraction-titration procedure and titratable acidity. The results presented in Figure 2 show that with increasing amount of lactic acid added to rancid milk, free fat acidity increased negligibly, whereas titratable acidity increased substantially. Since lactic-acid producing organisms are inhibited in rancid cream and milk *(4, 5, 21)*, the effect of lactic acid in rancid cream on the fat acidity is not a serious limitation.

Determination of free fat acidity in milk and cream of varying rancidity. The relationship between extent of rancidity and free fat acidity was determined by using rancid milk diluted with fresh milk in varying proportions to vary rancidity. Results from the proposed extraction-titration procedure were compared with titratable acidity and surface tension determinations. Figure 3

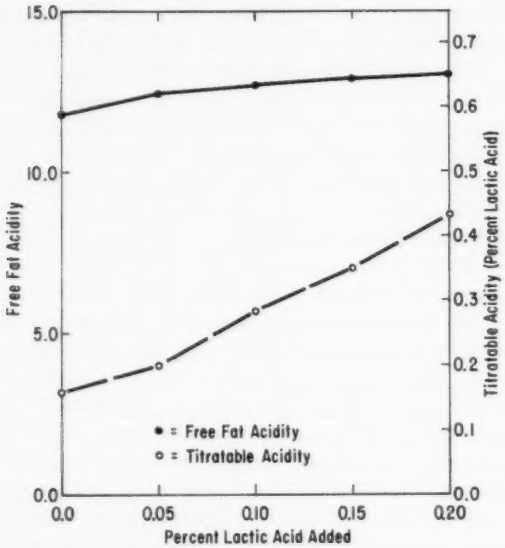

FIG. 2. Effect of lactic acid on the free fat acidity and titratable acidity of rancid milk.

shows that free fat acidity of milk as determined by the present procedure varies in direct proportion to the per cent rancid milk mixed with fresh milk.

Several experiments were carried out to establish an extraction-titration procedure for cream that would give the same free fat acidity as milk containing an equal amount of free fatty acids. A sample of rancid cream of 25% fat was

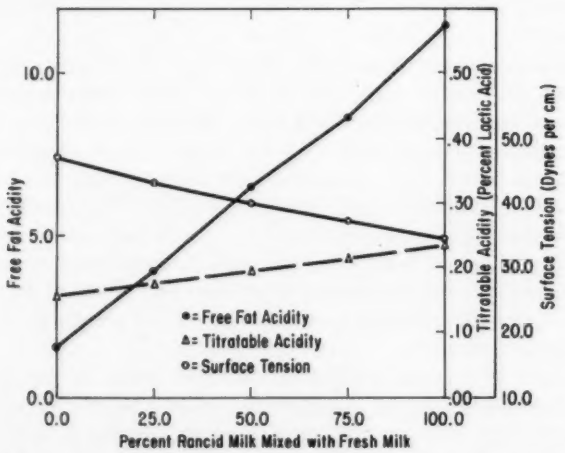

FIG. 3. Free fat acidity, titratable acidity, and surface tension of rancid milks varying in degree of rancidity.

diluted with fresh skimmilk to give samples with fat contents varying from 5 to 25%. When computing the free fat acidity, the extent of dilution of the degree of rancidity by the skimmilk was taken into consideration. It was observed that when the size of the sample was kept constant (at 10 g.) the free fat acidity decreased as the fat content of the samples increased. This indicated a lowering of the efficiency of the solvent extraction of fatty acids as the fat percentage of the samples increased. Therefore, the weight of the samples was so varied that the amount of fat was kept constant—corresponding with the fat content of 10 ml. of 4% milk. This maintained a constant free fat acidity, irrespective of the fat percentage of the cream samples. This latter procedure was thus adopted.

This established procedure for rancid cream was compared with the direct titration of fat obtained from the same cream by Johnson and Gould's extraction *(12)*—without the use of sulfuric acid—and by churning. Samples of varying rancidity were prepared by mixing rancid cream with fresh cream in varying proportions. The values for free fat acidity obtained by the present procedure were corrected for the titration value contributed by the nonfatty acid constituents of the creams. The latter value was determined on artificial emulsions of neutral fat passed through Amberlite IRA 400 and of fresh skimmilk, containing the same fat percentage as the corresponding cream. This value for the blank varied from 0.13 to 0.16 ml. of 0.025 *N* alkali per entire ether layer. Figure 4 reveals a linear relationship between rancidity and fat acidity as determined by all three procedures. The similar slopes obtained by the three methods can be explained by the fact that the same distribution of fatty acids existed in the samples of cream, which varied in degree of rancidity. It is evident that a higher sensitivity can be achieved by the method under study than by the titration of either the solvent extracted or the churned fats.

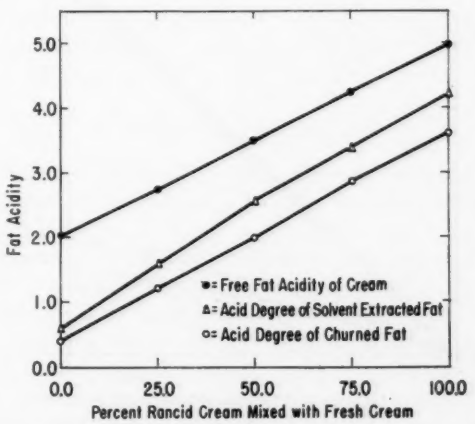

FIG. 4. Fat acidity of rancid creams as determined by proposed extraction-titration method and by titration of the fat obtained from the same creams by solvent extraction and by churning.

Higher initial values of free fat acidity for fresh cream were obtained by the method under study than by the other two procedures. Since these values are much higher than those usually obtained on fresh, churned fat, samples of fresh milk and cream from several sources were analyzed for free fat acidity by the method under study. Values ranging from 1.50 to 3.50 were obtained with an average of 2.01. This substantiates the findings of Thomas *et al. (22)* that the content of free fatty acids of fresh milk is much higher than has generally been accepted on the basis of acid degree values of churned fats. However, these authors did not consider the possible activation of "naturally active" lipase by cooling raw milk *(20)*. Further investigation is being made of variations of free fat acidity in freshly drawn milk.

Recovery of added fatty acids. The effectiveness of the proposed procedure to extract individual fatty acids was determined on artificial emulsions made with "acid-free" fat to which varying amounts of fatty acids were added and with fresh skimmilk, to give a 4% milk. In addition, rancid fat was mixed with fresh fat in varying proportions and then emulsified in skimmilk to give milks of varying rancidity. The free fat acidity obtained on controls made with "acid-free" fat was subtracted from that obtained on the corresponding rancid emulsions. The recoveries of individual fatty acids and the fatty acids from rancid fat were calculated from the slope of the line determined by the method of least squares, obtained by plotting free fat acidity of milk emulsions against the acid

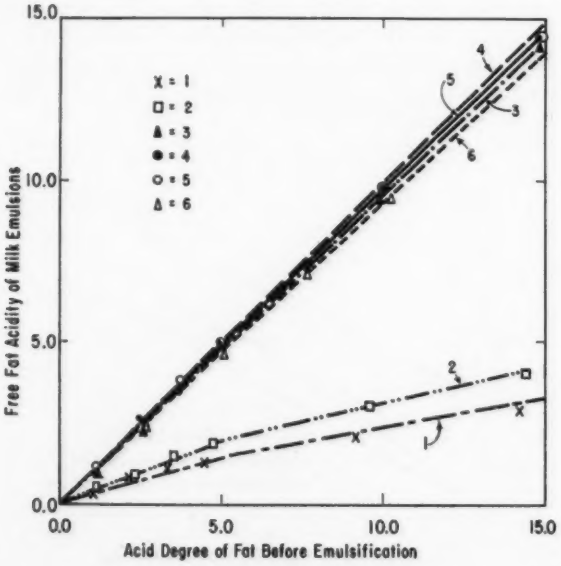

FIG. 5. Recovery of added fatty acids by proposed extraction-titration method in artificial milk emulsions (1 = butyric acid, 2 = caproic acid, 3 = palmitic acid, 4 = stearic acid, 5 = oleic acid, 6 = fatty acids from churned rancid fat).

degree of the fat before emulsification. The results of this experiment are presented in Figure 5. The percentage recovery calculated for butyric, caproic, palmitic, stearic, and oleic acids and the fatty acids from churned rancid fat are 29.3, 38.4, 95.6, 99.8, 97.3, and 93.2, respectively. The data for butyric and caproic acids were calculated on the basis of the initial slope in the range of acid degree from 0.00 to 5.00.

An attempt was made to compare the recovery of fatty acids according to whether they are added directly to milk or cream, as done by Johnson and Gould *(12, 13)*, or to fat before emulsification with skimmilk. This was only feasible for butyric acid, which is relatively soluble in water. The fatty acids above butyric acid in the homologous series are very slightly or not soluble in water and no homogeneous distribution could be obtained by their direct addition to milk. A recovery of 28.6% of butyric acid was obtained by the proposed extraction-titration procedure when this acid was added as a 0.25 *N* aqueous solution directly to milk. This recovery is equivalent to that obtained above by adding the acid to the fat phase of an emulsion. Therefore, butyric acid, when added directly to milk, appears to be distributed in the same way as when added to fat before emulsification with skimmilk.

TABLE 4
Reproducibility of results

Mean F.F.A. reading[a]	Standard deviation	95% confidence limits for mean F.F.A. reading
9.41	0.193	±0.0815
15.2	0.321	±0.153
22.8	0.477	±0.201

[a] For 24 determinations on each lot of rancid milk.

Reproducibility of results. The free fat acidity was determined twenty-four times on each of three lots of rancid milk by two technicians. The statistical analysis of these data is reported in Table 4. The narrow 95% confidence limits obtained for the mean free fat acidity values show that the results are highly reproducible.

SUMMARY AND CONCLUSIONS

An extraction-titration method for determining free fatty acids in rancid milk and cream is described. Factors affecting the recovery of fatty acids by this method were studied.

After evaluating various extraction methods for estimating free fatty acids in milk and cream, it is evident that no method is as yet available that permits quantitative recovery of all the fatty acids. It was demonstrated that the proposed extraction-titration method makes possible a 95 to 100% recovery of the high-molecular-weight fatty acids added and 52 to 58% of the low-molecular-weight fatty acids added (mixture of butyric, caproic, and caprylic—Tables 2 and 3). The method is simple and can be applied directly to milk and cream.

Although the information on specificity of lipase action in milk is only scanty in the literature, there is no evidence that either the high-molecular-weight fatty

acids or the low-molecular-weight fatty acids are exclusively hydrolyzed from milk fat. It appears, therefore, that the distribution of the products of hydrolysis of milk fat will be related to that of the fat's original fatty acid composition. This is supported by the similar fat constants obtained with ethanol fractions from fresh and rancid milk fats *(12)* and the analyses of steam-volatile and water-insoluble fatty acids in rancid fats *(8)*. The low-molecular-weight fatty acids (butyric, caproic, and caprylic) constitute only 14.7 mols per cent of the total fatty acids of milk fat *(11)*. Using the recovery values obtained by the proposed extraction-titration method, it can be estimated that about 90% of the total fatty acids released from milk fat at any given degree of hydrolysis can be determined by this method. Similar calculations applied to data obtained from churned fat *(19)* yield a value of approximately 65% for recovery of the fatty acids from milk fat.

In view of the limitations of the acid degree determination for churned fat, the validity of previous lipase studies based upon acid degree of churned fat has been questioned *(12)*. Although there are appreciable differences in recoveries of free fatty acids by various methods, the data presented in this paper indicate a linear relation between the extent of rancidity in cream and fat acidity as determined by the reported extraction-titration method and by the acid degree of solvent-extracted and churned fat. It is therefore concluded that previous lipase studies based upon acid degree of churned fat remain valid when this value was used for determining only the relative extent of rancidity.

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A PHOTOMETRIC METHOD FOR ESTIMATING THE LIPASE CONTENT OF MILK[1]

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The need for a lipase substrate which would be readily soluble in water, hydrolyzable by the lipase(s) of milk and would yield, on hydrolysis, a compound or radical capable of precise estimation by photometric procedures has been evident for some time. A photometric procedure making use of the esters of *p*-nitrophenol was suggested by Huggins and Lapides *(9)* but was criticized by Dirks and Boyer *(3)* on the grounds that hydrolysis of these esters was also catalyzed by nonenzymatic materials. Greenbank and Wright *(6)* outlined a colorimetric test for milk lipase using as substrate the fatty acid esters of *a*-naphthol. The writers believe that the esters of *p*-nitrophenol and *a*-naphthol lack one extremely important attribute of a good lipase substrate, namely, water solubility.

Beck *(1)* and Beck and Forster *(2)* prepared a crude form of the butyryl ester of mono-sulfonated phenol. They found this ester was readily soluble in water and as rapidly hydrolyzed by milk lipase as was tributyrin. Using titrimetric procedures they showed, in addition, that when tributyrin was used as substrate the rate of hydrolysis was greatly increased by continuous shaking of the reaction mixture, whereas shaking made no difference to the rate of hydrolysis of reaction mixtures containing the butyryl ester of sulfonated phenol.

Subsequent to publication of these reports a considerable, but unsuccessful, effort was made to prepare the butyryl ester of sulfonated phenol in a relatively pure form *(5)*. Attention was then directed to another class of compounds, the fatty acid esters of mono-sulfonated naphthol. These esters could not be obtained commercially, and search of the literature failed to reveal methods for their preparation. Syntheses of butyryl, caproyl, and caprylyl esters of 1-naphthol-4-sulfonic acid, 2-naphthol-8-sulfonic acid, and 2-naphthol-6-sulfonic acid were therefore attempted. Only with the latter compound were these attempts successful.

This paper describes methods used in synthesis of the butyryl, caproyl, and caprylyl esters of 2-naphthol-6-sulfonic acid. It reports on the preliminary testing of these substrates by photometric methods and suggests a lipase test for milk with the butyryl ester as substrate. It is believed that these esters offer sufficient promise as lipase substrates to warrant more extensive testing.

EXPERIMENTAL PROCEDURE

Synthesis of substrates. The butyryl, caproyl, and caprylyl esters of 2-naphthol-6-sulfonic acid (sodium salt) were prepared in the following manner: 0.1 *M*

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(24.6 g.) of 2-naphthol-6-sulfonic acid (sodium salt) were placed in a 500-ml. three-necked (standard taper) distilling flask. A stirring device was introduced through one neck, a second was closed with a glass stopper, and the third was fitted to a reflux condenser. The top of the condenser was connected, by way of a $CaCl_2$ drying tube, to a hydrogen chloride absorption trap. Sufficient appropriate acyl chloride (about 0.5 *M*) to make a slurry was added, and the mixture was warmed gently by means of an oil bath. Evolution of hydrogen chloride began immediately and became quite rapid within 5 minutes. The reaction mixture was heated, with agitation, until the acyl chloride refluxed slowly. Heating was continued for a period of about 45 minutes, at which time evolution of hydrogen chloride had practically ceased. After cooling, the mixture was dispersed in about 75 ml. of petroleum ether, filtered with suction, and the solid washed three more times with petroleum ether in a similar manner. After air drying the mixture overnight, the yield of crude product generally exceeded 90% of the theoretical. The crude product was purified by several recrystallizations from water. The final purified material was pure white crystalline and gave practically no color with Folin and Ciocalteu's phenol reagent. Yield of purified material was generally less than 50% for the butyryl ester but amounted to 65 to 70% for the caproyl and caprylyl esters. The relatively pure material from several succeeding preparations was combined, purified further by an additional recrystallization, thoroughly dried, and subjected to analysis. Table 1 presents results of these analyses.

TABLE 1

Fundamental composition and solubility of butyryl, caproyl, and caprylyl esters of 2-naphthol-6-sulfonic acid (sodium salt)

Ester	Composition (%)						Solubility in water at 25° C.
	Calculated			Found			
	C	H	S	C	H	S	
							g/100 g.
Butyryl	53.16	4.14	10.13	53.23	4.26	10.36	4.02
Caproyl	55.84	4.98	9.32	55.28	4.38	9.69	0.77
Caprylyl	58.05	5.68	8.69	57.09	5.20	9.00	0.18

Photometric estimation of 2-naphthol-6-sulfonic acid. The procedure used for determination of 2-naphthol-6-sulfonic acid was based on measurement of the blue color produced with Folin and Ciocalteu's phenol reagent in an Evelyn photoelectric colorimeter with the 515 filter. Figure 1 shows data for measurements made under the same conditions of substrate (butyryl ester), buffer, and milk (heated in water bath at 75° ± 5° C. for 10 minutes) concentrations, as are described below for the testing procedure. Milk proteins were also removed and color was developed and measured in the same manner as described for the testing procedure. It is evident from Figure 1 that the points fall essentially in a straight line. Hence, the optical density is directly proportional to concentration.

Stability of the esters. During purification of the esters it became apparent that an appreciable degree of hydrolysis was taking place at each recrystalliza-

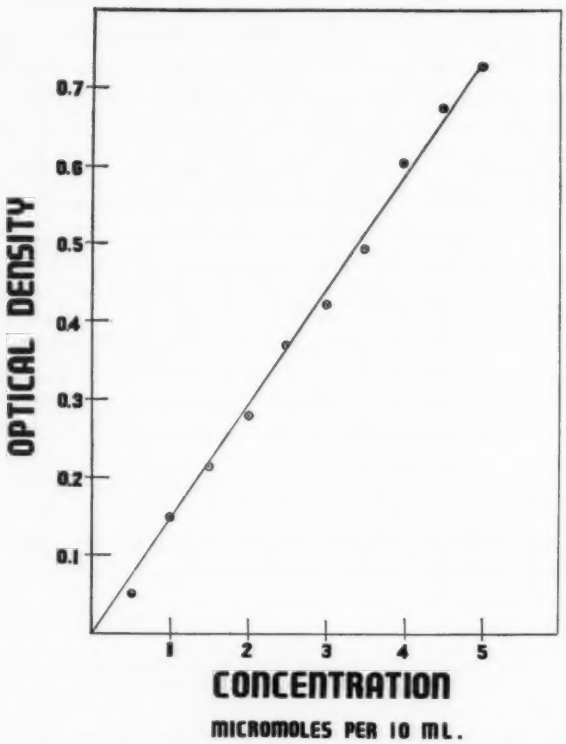

FIG. 1. Relationship between optical density and concentration of 2-naphthol-6-sulfonic acid.

tion. This complicated their purification and served as a warning that nonenzymatic hydrolysis of these esters must be considered in developing any testing technique in which they would be used as substrates. To obtain more information about their stability a study was made of the influence of pH on the degree of hydrolysis occurring during incubation for 1 hour at 40° C. in phosphate buffered solutions. Color was developed and measured in a manner similar to that described in a following section. Results are summarized in Table 2. It is evident

TABLE 2

Influence of pH on nonenzymatic hydrolysis of butyryl, caproyl, and caprylyl esters of 2-naphthol-6-sulfonic acid in phosphate buffered solutions

Butyryl		Caproyl		Caprylyl	
pH	% hydroylsis	pH	% hydroylsis	pH	% hydroylsis
6.29	0.16	6.17	0.18	6.28	0.42
7.02	0.67	6.96	0.57	6.95	0.44
8.07	4.95	8.19	5.21	8.00	4.03

Explanatory note. Ester and buffer concentrations were 0.001 *M* and 0.01 *M*, respectively. Incubation was for 1 hour at 40° C. All values are means of 5 trials.

that pH has a pronounced influence on the rate of nonenzymatic hydrolysis of these esters in aqueous solution; at pH 8.0 this rate is appreciable. It was not deemed necessary to repeat this work after details of the testing procedure had been worked out (incubation period of 15 minutes, ester and borate buffer concentrations, 0.006 *M* and 0.02 *M*, respectively) because use of the blank adequately compensates for nonenzymatic hydrolysis.

Substrate concentration (butyryl ester). Preliminary trials indicated that a substrate concentration of 50 μM per 10 ml. of reaction mixture provided for near maximum rates of hydrolysis of the butyryl ester. After many details of the testing procedure had been worked out, the experiment described below was undertaken to determine the most suitable substrate concentration to use in routine testing. Only the butyryl ester was studied in this experiment.

Hydrolysis of this ester at concentrations of 20, 40, 60, 80, and 100 μM per 10 ml. of reaction mixture was determined for nine samples of fresh, mixed herd milk, in accordance with the method described in the succeeding section. The resultant data are presented in Table 3 and mean values are plotted in Figure 2.

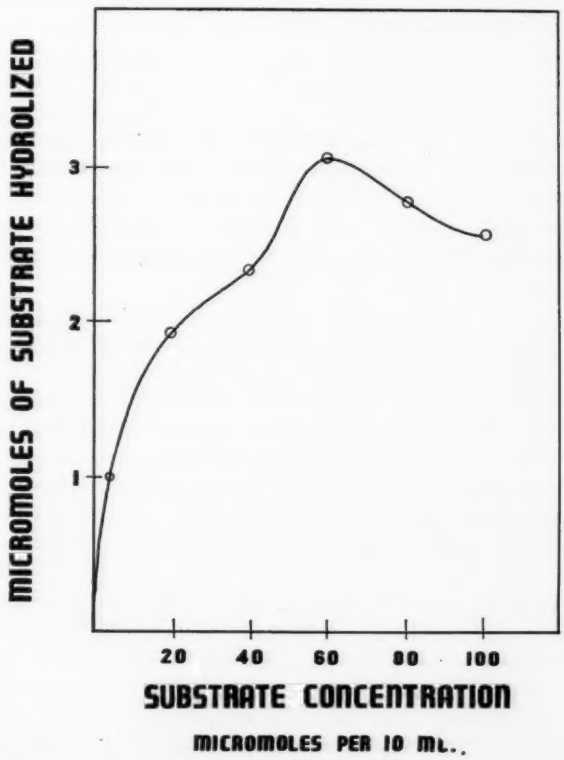

FIG. 2. Influence of substrate concentration on amount of substrate hydrolyzed.

TABLE 3

Micromoles of butyryl ester hydrolyzed at various substrate concentrations

Sample No.	Concentration of butyryl ester[a]				
	20	40	60	80	100
1	2.00[b]	2.59	3.18	2.96	2.66
2	2.22	2.41	3.36	2.92	2.52
3	2.00	2.34	3.42	2.65	2.35
4	1.64	2.00	2.62	2.24	2.16
5	1.59	2.00	2.24	2.22	2.21
6	1.74	2.26	2.68	2.68	2.47
7	1.72	2.14	2.48	2.50	2.38
8	1.90	2.26	2.65	2.60	2.62
9	2.56	2.92	4.83	4.04	3.56
Mean	1.93	2.32	3.05	2.76	2.55

[a] In micromoles per 10 ml. of reaction mixture.
[b] All values are means of duplicate determinations.

The curve shows a maximum at a substrate concentration of 60 μM, and Table 3 reveals that seven of the nine samples showed maximum hydrolysis at this substrate concentration. Sample 7 produced slightly more (0.02 μM) hydrolysis at a substrate concentration of 80 μM, and Sample 6 produced the same amount of hydrolysis at both 60 and 80 μM of substrate.

Another observation with respect to Figure 2 should be mentioned. That portion of the curve between 20 and 60 μM does not resemble a typical substrate concentration curve. Separate curves (not presented here) were plotted for each of the nine samples from the data in Table 3. Six of these nine curves show the same general shape in this region, and in four of these the deviation from normal is even greater.

Analysis of variance of the data in Table 3 indicates that the differences shown are significant. Mean square for substrate concentrations was 4.88 with an F value of 53.13 (1% point of F = 3.53).

Test for lipase of milk. Basically, the method, described in detail below, consists of incubating 10 ml. of a reaction mixture containing 0.5 ml. of whole milk and suitable concentrations of buffer and substrate for 15 minutes at 40° C. Enzyme action is then stopped and proteins are precipitated by addition of trichloroacetic acid. After filtration, a known volume of the clear filtrate is diluted in water and color is developed with Folin and Ciocalteu's phenol reagent *(4)*. The optical density of this solution is then compared with that of similar solutions from a suitably prepared blank and standard. Lipase activity in the blank and standard is prevented by using milk in which the enzyme has been destroyed by heat.

A sample of milk is tested for lipase in the following manner: Set up a series of four, or more, test tubes (25 × 150 mm.) as shown in Table 4. Add water, buffer, and standard to the tubes, in that order and as rapidly as is consistent with accuracy. Place tubes in a water bath (at 40° C.) and allow 5 minutes for temperature equalization. Add the milk at 1-minute intervals, adding heated milk to the blank and standard tubes and raw milk to the unknown tubes. Add substrate (previously warmed to 40° C.) at 1-minute intervals and record the

TABLE 4
Details of setup for a single lipase determination

Tube No.	Purpose of tube	ml. H_2O	ml. Buffer[a]	ml. Standard[b]	Milk		ml. Substrate[d]
					ml.	Trt.[c]	
1	Blank	3.5	2.0	0	0.5	H	4
2	Standard	0.5	2.0	3	0.5	H	4
3	Unknown	3.5	2.0	0	0.5	R	4
4	Unknown	3.5	2.0	0	0.5	R	4

[a] 0.1 molar, Clark and Lubs' borate *(7)*, pH 8.7.
[b] 0.001 molar, 2-naphthol-6-sulfonic acid (sodium salt).
[c] Treatment. H = heated (5 to 10 ml. heated in water at 75° ± 5° C. for 10 min.) R = raw.
[d] 0.015 molar, butyryl ester of 2-naphthol-6-sulfonic acid (sodium salt).

time of addition as the start of the incubation period. After 15 minutes of incubation, at 1-minute intervals, add 2 ml. of trichloroacetic acid solution (140 g. per 500 ml. of solution) and mix thoroughly. Allow at least 20 minutes for protein precipitation, mixing several times during this period. Filter, at 1-minute intervals, through 11-cm. Whatman No. 2 filter paper. Again at 1-minute intervals, transfer 3 ml. of the clear filtrate to a test tube (25 × 150 mm.) containing 10 ml. of water, and mix. Develop color, at 1-minute intervals, in the diluted filtrate solutions in the following manner: Add 1 ml. of diluted (1 part reagent to 2 parts 5% sodium hexametaphosphate) Folin and Ciocalteu's phenol reagent (Harleco) and 3 ml. of 20% sodium carbonate solution, and mix. Allow 20 to 30 seconds for the color reaction to proceed, then heat in a boiling water bath for exactly 60 seconds. Cool immediately in ice water for 2 minutes and in water at room temperature for 4 minutes. Experience indicates that the duration of heating and the rate of cooling are critical. This is particularly true of the heating period.

Determine per cent transmittance of the standard and unknown solutions in a photoelectric colorimeter with the instrument set at 100% transmittance for the blank. Very satisfactory results have been obtained in this laboratory with an Evelyn instrument, with the No. 515 filter and the 10 ml. aperture. Determine the optical density, D, values for the standard and unknown solutions and from the optical density of the standard and its known concentration, c, determine the constant, k, in the equation

$$D = kc$$

By using the value of k determined as above and the D values for the unknown solutions, the concentration of 2-naphthol-6-sulfonic acid in the unknowns, which has resulted from enzyme hydrolysis, may be calculated from the equation presented. If c is expressed in terms of micromoles per 10 ml. of reaction mixture, the values obtained for c also represent the amount of ester hydrolyzed by the enzyme.

Definition of a lipase unit. The authors propose to define a lipase unit as: That amount of lipase which will hydrolyze 1 μM of the butyryl ester of 2-naphthol-6-sulfonic acid (sodium salt) when the reaction mixture is 0.006 molar with

respect to this substrate, 0.02 molar with respect to Clark and Lubs' borate buffer, has a pH of 8.6, and is incubated at 40° C. for 15 minutes. If the test for lipase is performed as described above, results may be expressed in terms of lipase units per milliliter of milk by multiplying the c values by two.

Function of the heated milk blank. The function of the heated milk blank, in the method described above, is to compensate for color producing compounds in the reagents and milk and for nonenzymatic hydrolysis of the substrate taking place during conduct of the test. This is accomplished automatically by adjusting the photoelectric colorimeter to 100% transmittance for the blank. Decreases in transmittance for the standard and unknown tubes are then due only to added 2-naphthol-6-sulfonic acid (in the standard) or to that produced by enzymatic hydrolysis of the ester (in the unknowns).

This technique assumes that the constant, k, obtained with a heated milk blank and standard, is valid to use as a basis of comparison with the unknown tubes containing raw milk. To determine the validity of this assumption, k values were made in triplicate by three different methods on 11 samples of mixed herd milk. The three methods which were compared were *(a)* raw milk added to blank and standard tubes after incubation and addition of the trichloroacetic acid, *(b)* pasteurized milk (63° ± 1° C. for 30 minutes) added as described in the outline of the lipase test, and *(c)* heated milk (75° ± 5° C. for 10 minutes) added in the same manner. In all other respects the method followed was identical to that described for the regular lipase test. Mean values of k for the 33 observations by each method were 0.1331 for raw milk, 0.1358 for pasteurized milk, and 0.1360 for heated milk. Analysis of variance showed that these means were not significantly different. ($F = 1.70$, 5% point of $F = 3.11$). These observations establish the validity of the assumption that k is a constant for a given sample of milk regardless of whether raw, pasteurized, or heated (75° ± 5° C.) milk is used in the blank and standard tubes.

Analysis of variance of the data obtained in this experiment also showed that there is a significant difference in the k values for different samples of milk. ($F = 2.46$, 5% point of $F = 1.97$). No doubt much greater differences between samples would have been observed had they been obtained from individual cows rather than from the mixed herd milk.

Accuracy of the method. The experiment described in this section was undertaken to determine the reproducibility of results obtained with the testing procedure described above. Duplicate lipase tests were made on ten samples of mixed, fresh herd milk. Each test consisted of one blank, one standard, and two replicate unknowns. All tubes for the two tests were set up and run through the various steps at one time (at 1-minute intervals). This meant that the second test followed the first by an interval of 4 minutes. The testing procedure followed exactly the method described above for the lipase test.

Data from this experiment together with pertinent statistics are presented in Table 5. Results are expressed in terms of micromoles of substrate hydrolyzed. Examination of Table 5 reveals that differences between replicates of a single test, on any one sample, are small. The standard deviation for a single replicate

TABLE 5

Comparative accuracy of the lipase test using the butyryl ester

Sample No.	Micromoles of substrate hydroylzed				
	Test No. 1		Test No. 2		
	Rep. 1	No. 2	Rep. 1	No. 2	Sample means
1	6.58	6.46	6.38	6.43	6.462
2	5.60	5.47	5.56	5.65	5.570
3	4.95	4.91	4.79	4.82	4.868
4	4.27	4.37	3.92	3.95	4.128
5	5.50	5.64	5.47	5.43	5.510
6	4.80	4.84	4.76	4.70	4.775
7	6.00	5.84	6.03	5.98	5.962
8	6.06	6.06	5.78	5.83	5.932
9	5.64	5.60	5.42	5.38	5.510
10	5.34	5.38	5.18	5.02	5.230
Test means	5.466		5.324		

Standard deviation for a single replicate = 0.10.
Standard error of mean of a single test = ±0.07.

was found to be 0.10, and the standard error of the mean of two replicates was ±0.07. Examination of data in Table 5 also reveals that the mean of Test 1 for all samples was 5.466 μM of substrate hydrolyzed, whereas the corresponding value for Test 2 was 5.324, a difference of 0.142 μM. Analysis of variance of these data indicated that this difference is significant (F = 21.30, 1% point of F = 7.64). As would be expected, the large differences between sample means was quite significant (F = 194.04, 1% point of F = 3.23).

DISCUSSION

Little difficulty was experienced in synthesizing and purifying the esters used in these experiments after basic details of the method had been worked out. Their solubility in water increases markedly as the temperature is raised, making crystallization from water a convenient method of purification. It was apparent, however, that they hydrolyzed more rapidly at elevated temperatures. To compensate, the time at which their solutions were held at the higher temperatures was kept at a minimum. Other, higher molecular weight, esters in this series were not prepared because it was assumed, on the basis of experience gained with the first three, that they would not possess sufficient solubility to be useful as substrates. It would appear, however, that their synthesis could be accomplished by essentially the same method as described for the butyryl, caproyl, and caprylyl esters.

The method of lipase estimation proposed herein makes use of the butyryl ester of 2-naphthol-6-sulfonic acid as substrate. Either of the two other esters may be used with satisfactory results, as has been demonstrated repeatedly. Because of their lower solubility (Table 1), however, it may not be possible to use them at the same recommended concentration (0.006 molar). Solutions of these esters in water are prepared in this laboratory and stored in amber glass bottles at 0° to 4° C. for about a week. Under these conditions hydrolysis, as measured

in terms of an increase in color of the blank, does not appear to be excessive. However, the actual amount of hydrolysis taking place during such storage has not been determined. It would simplify the testing procedure if substrate and buffer could be made up as one solution, but the excessive rate of hydrolysis of these esters at the pH of the buffer solution would seem to preclude this.

Clark and Lubs' borate buffer *(7)* was selected on the basis of previous experience in this laboratory *(1)*. Two ml. of a 0.1 molar solution of this buffer with a pH of 8.7 will provide the desired concentration (0.02 molar) and pH (8.6) in 10 ml. of reaction mixture containing 0.5 ml. of milk. A pH of 8.6 for the reaction mixture was selected on the basis of previous reports *(1, 10)* and on the finding that the butyryl ester was hydrolyzed most rapidly, by milk lipase, at this pH.

Previous reports *(1, 10)* also established an incubation temperature of 40° C. as being satisfactory. The incubation time and milk concentration can be varied. However, an incubation time of 15 minutes, coupled with 0.5 ml. of milk in 10 ml. of reaction mixture, will provide for optical density values within the most accurate range of the photometer and, moreover, will reduce the amount of nonenzymatic hydrolysis to a minimum. This is the incubation time and milk concentration recommended for routine testing.

The blank is a fundamental part of this method of lipase estimation. It compensates for any nonenzymatic hydrolysis occurring between the time of substrate addition and color development. A separate blank for each milk sample is advisable because some component of milk contributes to the color and this component varies from sample to sample. The blank is set up and carried through all steps of the method in a manner similar to the unknowns with the exception that the milk used has been heated to inactivate the lipase.

The standard tube is identical to the blank except that a part of the water is replaced by a solution of 2-naphthol-6-sulfonic acid (sodium salt) to give the desired concentration in the reaction mixture. For precise work the concentration of sulfonated naphthol in the standard should approximate that released by enzymatic hydrolysis of the ester in the unknowns. A standard containing 2 to 4 μM of the naphthol has proved satisfactory for routine testing of milk. The 2-naphthol-6-sulfonic acid used in all of the work reported was prepared from Eastman No. T399 2-naphthol-6-sulfonic (sodium salt, Techn) by repeated recrystallization from water until a pure white product was obtained.

These esters of sulfonated naphthol are subject to nonenzymatic hydrolysis as previously mentioned. It has been established that the rate of this hydrolysis is influenced by pH (Table 1). One would expect that other factors such as temperature, concentration, and time might also influence the amount of this type of hydrolysis taking place during the conduct of the test. For these reasons all tubes making up a single test for lipase must be handled in precisely the same manner and on a definite time schedule. The authors have found it possible to adopt a 1-minute time interval for each of the steps in the method from addition of the milk to development of color. The last step is critical with respect to the duration of heating and speed of cooling.

A part, at least, of the lipase of fresh milk appears to be quite unstable. Others *(8, 10)* have referred to this property and in one instance *(8)* found that deaeration by ebullition with nitrogen resulted in a marked degree of stabilization. Under the previous section, *Accuracy of method*, it was shown that duplicate lipase tests, on fresh milk, failed to agree. The second test was significantly lower than the first, although the time interval between the two tests was only 4 minutes. Other data, not reported here, indicated that the differences became greater as the interval between duplicates was increased. This lack of stability of milk lipase poses a difficult problem when one wishes to determine the concentration of this enzyme in milk at the time it is drawn. It is not convenient to make the test in the barn, nor is it desirable to rush to the laboratory with each sample. This problem is presently under study at this station.

The technique of lipase estimation by the method described herein is necessarily complicated by three considerations. These are: *(a)* The proposed substrates undergo appreciable nonenzymatic hydrolysis during the incubation period, *(b)* different samples of milk contribute varying amounts of color producing compounds, and *(c)* the lipase content of fresh milk decreases rather rapidly. These considerations make it necessary to carry a separate blank and standard for each milk sample being examined. They also make necessary the adoption of a strict time schedule for the conduct of each operation of the testing procedure. The authors believe, however, that in spite of these complicating factors the method may be of distinct value in studying the physical and chemical properties of milk lipase and in determining the influence of various practical factors upon the lipase content of milk. It would also appear that the method, with suitable modifications, would have value in studying the lipase content of other biological material such as blood, lymph and various tissue homogenates.

SUMMARY

The butyryl, caproyl, and caprylyl esters of 2-naphthol-6-sulfonic acid (sodium salt) have been synthesized and purified. These esters, particularly the butyryl, possess a reasonable degree of water solubility and are all hydrolyzed by some component of raw milk, presumably the lipase. 2-naphthol-6-sulfonic acid (sodium salt), one of the products of hydrolysis of these esters, can be estimated photometrically by using Folin and Ciocalteu's phenol reagent to develop color. A photometric method for estimating the lipase content of whole milk is presented which makes use of these esters as substrates and in which color is developed with the above mentioned reagent.

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THE SELECTIVE RELEASE OF VOLATILE ACIDS FROM BUTTERFAT BY MICROBIAL LIPASES

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The lipases of microorganisms have been studied extensively from the aspects of occurrence in individual species and factors affecting enzyme production and activity. These lipases exhibit different patterns of substrate hydrolysis which can be attributed in part to the source of the enzyme and the nature of the substrate *(4, 6, 13, 14)*.

Specifically, the lipases of *Penicillium roqueforti* and *Aspergillus niger* showed distinct differences in cleavage products when an equimolar mixture of tributyrin and tricaprylin was used as substrate. The *P. roqueforti* lipase released butyric and caprylic acids in the ratio of 3:1, whereas the same acids released by *A. niger* lipase were in a 1:4 ratio *(13)*. The lipases of *P. roqueforti* also exhibited greater relative specificity for triglycerides than for ethyl esters, and tributyrin was more readily hydrolyzed than butterfat *(4)*. *Geotrichum candidum* lipase hydrolyzed natural fats, such as olive oil and butterfat, more readily than synthetic triglycerides, monoglycerides, and fatty acid esters of glycols *(6)*.

Richards and El-Sadek *(12)* reported that molds produce a greater total acidity in butterfat than bacteria under identical conditions. Lipolytic bacteria produced relatively less volatile acids than molds but slightly more solid fatty acids. These investigators also observed that of the volatile acids produced, the bacteria produced a greater portion of butyric and caproic acids than the molds. Fouts *(2)* reported that rancidity and the acid value of butterfat in commercial butter were not correlated and that fat in cream which had supported the growth of *Oospora lactis (G. candidum)* for 6 days contained only 1.9% of the total fat acidity as volatile acids. In contrast, other microorganisms, including *Mycotorula lipolytica (Candida lipolytica)* produced appreciable amounts of volatile acids, which ranged from 8.4 to 14.7% of the total fat acidity.

Although relative substrate specificities or preferences of some microbial lipases have been described, little is known of the specificity of these lipases in relation to the selective release of the component fatty acids of complex natural fats. The purpose of this report is to present data relative to the identity of the C_4-C_8 fatty acids released from butterfat by cell-free lipases from *G. candidum* and other lipolytic species. The data indicate that microbial lipases differ in their capacity to free butyric, caproic, and caprylic acids from butterfat.

METHODS AND MATERIALS

Bacteriological. The identity of the cultures and the sources from which they were obtained are shown in Table 1. The media used for lipase production included Protolysate broth *(6)* for *G. candidum* and the *Penicillium* species, pep-

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TABLE 1
Sources of experimental cultures

Culture	Identification	Source
Candida lipolytica	840, 843, 845, 846, 848	F. E. Nelson, Dairy Industries Dept., Iowa St. Coll., Ames.
Candida lipolytica	I	Dept. of Dairy Science, Univ. of Ill., Urbana.
Geotrichum candidum	13, 73A, 74B, 75C, 76D 77E, 78F, 79G, 80H	Dept. of Dairy Science, Univ. of Ill., Urbana.
Penicillium roqueforti	P-108-1, P_{12}-1M_2	J. J. Jezeski, Dept. of Dairy Husbandry, Univ. of Minn., St. Paul.
Penicillium camemberti	K	R. V. Hussong, Research Lab., Kraft Foods Co., Glenview, Ill.
Achromobacter lipolyticum	1 X B-1	R. V. Hussong, Research Lab., Kraft Foods Co., Glenview, Ill.

tone-beef extract broth *(9)* for *C. lipolytica*, and a phosphate-buffered peptone solution *(5)* for *Achromobacter lipolyticum*.

Lipase preparations. Cell-free filtrates from cultures incubated at 26° C. for 3 days were used as the sources of lipase. *G. candidum* lipase was obtained by filtering the cultures through asbestos-matted Gooch crucibles to remove the mycelia. The supernatant liquid of centrifuged cultures of *C. lipolytica* and *A. lipolyticum* was the source of lipases from these species. The lipases of the *Penicillium* species were prepared by homogenizing the mycelia in a cold Pyrex tissue homogenizer and recombining the filtrate and homogenate.

Lipase assay. Lipase activity was estimated by titrating the fatty acids liberated at 32.6° C. from a buffered substrate prepared and handled as previously described *(6)*. *G. candidum* reaction mixtures were buffered at pH 6.0 *(6)*, those of *C. lipolytica* at pH 6.5 *(9)*, and those of the other test organisms at pH 7.2 *(3, 5)*.

Chemical. The volatile acids were separated from the reaction mixtures by the steam distillation procedure proposed by McAnnally *(3)*. A 10-g. aliquot was diluted with 50 ml. of a saturated magnesium sulfate solution containing 25% concentrated sulfuric acid, and the total volume was made up to 100 ml. with distilled water. This mixture was shaken, allowed to stand for 5 minutes, and filtered. A 50-ml. aliquot of filtrate was subjected to constant volume steam distillation, and 50 ml. of distillate was collected. Preliminary trials indicated an 89.79 ± 2% recovery of known concentrations of butyric acid added to reaction mixtures.

The volatile acidity of the distillate was measured by titrating a 10-g. aliquot to a phenolphthalein end point with 0.1 *N* alcoholic KOH delivered by a microburette. Excess ammonium hydroxide was added to the remaining distillate, which was then placed on a steam bath and concentrated under alkaline conditions to volume of approximately 1 ml.

The separation and identification of the free saturated C_4-C_8 fatty acids in 0.05-ml. aliquots of concentrated distillates was accomplished by paper partition

chromatography with resdistilled *n*-butanol saturated with an equal volume of 1.5 *N* ammonia *(11)*. The indicator solution was 0.04% (w/v) bromeresol purple in a 1:5 (v/v) dilution of formalin in ethanol adjusted to pH 5.0 with 0.1 *N* sodium hydroxide. The Rf values of control acids were essentially those reported by Reid and Lederer *(11)*. Preliminary trials demonstrated that a concentration of acid as low as 4.8γ on the paper was detected by this procedure.

All trials and all analyses were run at least in duplicate and mean values are shown. Titration values of controls containing heated lipase did not change during incubation.

RESULTS

The data in Table 2 show the relative activity of the lipases of *C. lipolytica* and *G. candidum* on three widely different substrates. Tributyrin was more readily hydrolyzed than olive oil and butter oil by the *C. lipolytica* lipase. In contrast, olive oil and butter oil were hydrolyzed by the *G. candidum* lipase, whereas tributyrin was not appreciably attacked. It has been shown in previous work *(6)* that tributyrin may be hydrolyzed by *G. candidum* lipase but to a smaller degree than either olive oil or butter oil. These data suggest that, although there may be quantitative differences in the extent of lipolysis between experiments, qualitatively olive oil is more readily hydrolyzed than butter oil by *G. candidum* lipase and that these substrates support greater lipolysis than tributyrin.

The wide differences in the extent of lipolysis of olive oil, butter oil, and tributyrin by *G. candidum* lipase suggest a preference of this enzyme for long-chain fatty acid linkages of butterfat and olive oil, and that the short-chain fatty acid esters may be less susceptible to hydrolysis. Thus, the relative amount of specific fatty acid esters may be a limiting factor in the lipolysis of butter fat by *G. candidum* lipase.

A series of experiments was conducted to test the effects of the addition of butter oil, lipase, and buffer added separately to reaction mixtures in which lipolysis had approached a maximum. Double volume reaction mixtures were

TABLE 2

Lipolysis of selected substrates by cell-free culture filtrates from C. lipolytica *and* G. candidum

Culture	Substrate titration values[a]					
	Olive oil		Butter oil		Tributyrin	
	(6 hr.)	(24 hr.)	(6 hr.)	(24 hr.)	(6 hr.)	(24 hr.)
C. lipolytica I	1.48	2.56	0.89	1.51	2.05	4.29
C. lipolytica 846	1.65	2.69	0.86	1.60	4.28	5.85
C. lypolytica 848	1.26	2.53	1.47	2.32	5.60	7.81
G. candidum 77E	0.29	0.83	0.20	0.36	0.00	0.07
G. candidum 78F	0.21	0.65	0.10	0.27	0.00	0.00
G. candidum 79G	0.32	0.93	0.10	0.29	0.00	0.05

[a] Net mean duplicate values of duplicate reaction mixtures expressed as ml. of 0.1 *N* KOH per 10 g. aliquot after 6- and 24-hour incubation. Reaction mixtures contained 44 g. buffered agar, 1 g. substrate, 5 g. culture filtrate, and 0.3 ml. toluene.

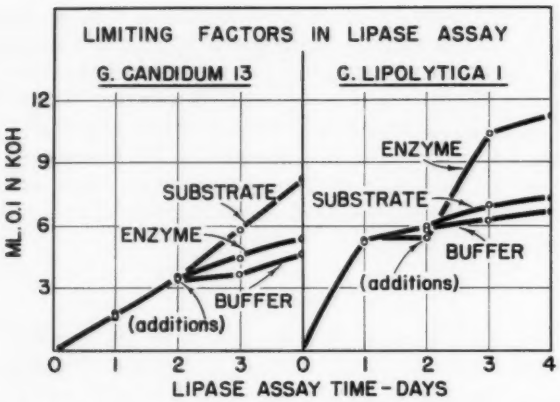

FIG. 1. Limiting factors in lipase assay. Assay emulsions contained 80 g. buffered agar (pH 6.0 and pH 6.5 for *G. candidum* and *C. lipolytica* lipases, respectively), 10 g. butter oil, and 10 g. lipase solution. After 2 days at 32.5° C. 10 g. butter oil, 10 g. enzyme solution, or 10 g. buffer solution was added as indicated and emulsion reincubated. Data are calculated from mean titration values for 10 ml. aliquot.

prepared and incubated, and aliquots were titrated at 24 and 48 hours. After 48 hours the additions indicated were made, and the course of lipolysis was followed for an additional 48 hours.

The data from these experiments, represented in Figure 1, show that supplementation of the *G. candidum* reaction mixture with additional enzyme did not appreciably alter the course of lipolysis. However, the supplementary addition of butter oil resulted in increased titration values during the second incubation period. The addition of more substrate to *C. lipolytica* reaction mixtures was without marked effect, whereas the addition of more enzyme resulted in resumption of lipolysis of the substrate already present. These data further suggest that the relative amounts of specific susceptible fatty acid linkages may be a factor limiting the extent to which butter oil is hydrolyzed by *G. candidum* lipase and suggest differences in R-group specificity of microbial lipases.

The concentrations of titratable acidity in distillates of reaction mixtures containing lipases from different species also suggest that these lipases differ in their ability to cleave volatile fatty acids from butter oil. In *C. lypolytica* lipase reaction mixtures prepared from six strains the titratable volatile acidity of distillates ranged from 0.05 to 0.22 ml. of 0.1 *N* KOH per 10 ml. aliquot, whereas those from nine cultures of *G. candidum* ranged from 0.01 to 0.04 ml. Total fat acidity in 10-g. aliquots of reaction mixtures ranged from 1.48 to 6.79, and 2.63 to 6.13 ml. of 0.1 *N* KOH for the six *C. lipolyticum* and nine *G. candidum* cultures, respectively.

The chromatographic separation and identification of the volatile C_4-C_8 fatty acids contained in concentrated steam distillates of butter-oil-lipase reaction mixtures are summarized in Figure 2. As can be seen, volatile fatty acids were

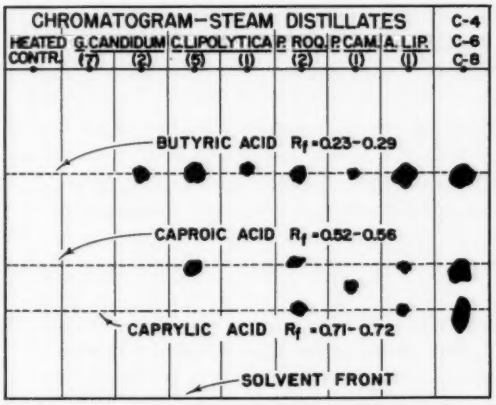

FIG. 2. Chromatogram—steam distillates. A schematic representation of fatty acids detected in concentrated steam distillates from lipase-butterfat assay emulsions incubated 3 days at 32.5° C. Figures in parentheses under each species denote number of strains tested.

not detected in seven *G. candidum* reaction mixture distillates, and only butyric acid was detected in distillates from *G. candidum* 74-B and 75-C reaction mixtures.

Butyric acid was present in all trials with *C. lipolytica* lipase, and caproic acid also was found in distillates from all *C. lipolytica* reaction mixtures except that containing the lipase from strain 840.

The lipases of the *P. roqueforti* and *A. lipolyticum* cultures tested liberated butyric, caproic, and caprylic acids from the butter oil substrate. Butyric acid and an unidentified acid, which consistently migrated to an Rf value midway between the C_6 and C_8 controls, were encountered in *P. camemberti* reaction mixtures.

DISCUSSION

The absence of appreciable volatile acidity in butterfat which has undergone extensive lipolysis has led to the belief that certain lipolytic molds consume the volatile fatty acids as rapidly as they are released *(2, 8)*.

Fouts *(1, 2)* reported that 1.5-2.4% of the total fat acidity of cream cultures of *O. lactis* was volatile acidity, as compared to values of 8.1-8.9, 13.1-18.9, 10.6-12.5, and 10.7-12.0 for *M. lipolytica*, *Pseudomonas fluorescens*, *A. lipolyticum*, and *Alcaligenes lipolyticus*, respectively. In this connection, Purko *et al. (10)* observed that the volatile acidity of cream supporting pure cultures of *G. candidum* did not change during an incubation period in which the water insoluble free fatty acids increased from a value of 200 mg. to 30,000 mg. per 100 g. of fat.

Salts of some volatile fatty acids in artificial media are utilized by cultures of *G. candidum (2, 7)*. However, the release of such acids from butterfat by cell-free lipase of *G. candidum* should be demonstrable if fatty acid utilization is a valid explanation of the conspicuously low volatile acidities encountered in butterfat hydrolyzed by growing cultures of *G. candidum*.

The experimental data presented in this report indicate that differences in microbial lipase specificities may account for the differences in volatile acidity encountered in butterfat that has been hydrolyzed by *G. candidum* and other lipolytic organisms.

During the course of this investigation several categories of data pertaining to the question of cell-free lipase specificity have been obtained. The relative substrate specificity patterns in Table 2 suggest that the *G. candidum* lipase has a predilection for long-chain fatty acid esters, such as those in olive oil, whereas the short-chain esters may not be attacked. In contrast, the *C. lipolytica* lipase hydrolyzes short-chain triglycerides more readily than higher fatty acid triglycerides.

The concentrations of volatile acidity in distillates of reaction mixtures containing cell-free lipases also suggest that the above lipases differ in their ability to cleave volatile fatty acids from butterfat. Similarly, the data shown in Figure 1 suggest that the relative amount of specific susceptible fatty acid esters may be one of the factors limiting the extent to which butterfat is hydrolyzed by *G. candidum* lipase. The *C. lipolytica* lipase was not limited in the same manner.

The chromatographic inventory and identification of volatile fatty acids released from butterfat by cell-free microbial lipases also indicate that these lipases possess certain fatty-acid R-group specificities. These data, summarized in Figure 2, show that lipases of seven of nine *G. candidum* cultures did not release detectable quantities of volatile fatty acids from butterfat. The lipases of the two other cultures liberated butyric acid as the only detectable acid. All of the *C. lipolytica* lipases tested released butyric acid, and five of the six cultures also released caproic acid. The lipases of the *P. roqueforti* and *A. lipolyticum* cultures tested hydrolyzed butyric, caproic, and caprylic acids from the substrate.

SUMMARY

Relative substrate specificity patterns, differences in volatile fat acidity of hydrolyzed fat, substrate limitations in the course of the lipolytic reaction, and differences in chromatographic analysis of distillates from hydrolyzed fat indicate that cell-free microbial lipases differ in their ability to release volatile fatty acids from butterfat. Some strain variations were evident.

The lipases of seven of nine cultures of *G. candidum* did not release detectable quantities of volatile fatty acids from butterfat. Two cultures freed butyric acid only.

C. lipolytica lipases hydrolyzed butyric acid from the butterfat and five of the six culture lipases also released caproic acid.

Butyric, caproic, and caprylic acids were found in distillates of reaction mixtures containing butterfat and the lipases of either *P. roqueforti* or *A. lipolyticum*.

Butyric acid and an unidentified acid which consistently migrated to an Rf value midway between the caproic and caprylic controls were encountered in *P. camemberti* lipase-butterfat reaction distillates.

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METABOLISM OF BOVINE SEMEN. II. QUALITATIVE ANAEROBIC CATABOLISM OF GLUCOSE-C^{14} BY BOVINE SPERMATOZOA[1,2]

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The formation of lactic acid as a direct product of glycolysis by spermatozoa is almost universally accepted *(1, 10)*. Frequently, however, the lactic acid formed during a trial does not account quantitatively for the sugar which disappears *(1, 7, 11)*. Such results may reflect either the existence of alternate pathways of metabolism which involve the formation of less than 2 *M* of lactic acid per mole of hexose consumed, or the formation of 2 *M* of lactic acid per mole of hexose consumed and the subsequent breakdown of lactic acid into other compounds.

Studies were undertaken in this laboratory to determine the metabolic patterns of bovine spermatozoa under various conditions; this report presents results of an investigation of the major products of the anaerobic dissimilation of glucose-C^{14}.

METHODS

A series of experiments was conducted in order to detect and identify products of glucose catabolism by bovine spermatozoa. Semen for the trials was obtained with the artificial vagina from mature Holstein bulls. The experimental procedure was begun within 2 hours after the collection of semen samples. Pooled ejaculates from three to four bulls were used in each trial; individual ejaculates included in the pool had an initial motility of at least 50% and a concentration of no less than 10^9 spermatozoa per milliliter. The spermatozoa were washed twice to remove seminal plasma *(6)* and suspended in calcium-free Ringer-phosphate solution, pH 7.4; 2.5×10^9 total spermatozoa were placed in each incubation flask. The incubation flasks were similar to those described by Bloom *et al. (3)*. Two-tenths ml. of 10 N sulfuric acid was added to each control flask to inactivate the spermatozoa. Five thousand units of sodium penicillin was added to each flask to prevent bacterial interference, although bacterial growth should not affect results until after 3 hours at 37° C. *(9)*. Ringer-phosphate solution was added in amounts such that the final volume in each flask after the addition of glucose-C^{14} would be 5 ml. Flasks were gassed with nitrogen and equilibrated to 37° C. in a water bath. Uniformly labeled glucose-C^{14} was dissolved in Ringer-phosphate solution and 0.5 ml. containing 8.5 to 8.7 μc. was added to each flask. Flasks were incubated under nitrogen at 37° C.

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After 2.5 hours of incubation, 1 ml. of 30% potassium hydroxide was injected into the center compartment of the incubation flask by means of a small gauge needle and syringe. The compartment contained a roll of filter paper to facilitate the absorption of carbon dioxide. At 3 hours the contents of the incubation flask were deproteinized with cold 10% trichloroacetic acid. The filter paper and potassium hydroxide were transferred to a 50-ml. centrifuge tube; the center compartment was washed several times with carbon dioxide-free water, and the washings were added to the centrifuge tube. Five ml. of 0.25 *N* barium hydroxide containing 2% barium chloride was added to convert the trapped carbon dioxide to barium carbonate. The precipitated barium carbonate was washed in the centrifuge, first with carbon dioxide-free water, then with alcohol and ether (3:1). The precipitate was suspended in alcohol-ether, filtered onto a tared filter paper disk, washed with alcohol-ether, and dried, and the weight and radioactivity were determined. Samples were assayed for radioactivity in a windowless flow gas G.M. counter in conjunction with a decade scaling unit. Radioactivities were corrected for self-absorption to a constant thickness of 1 mg. per square centimeter.

The trichloroacetic acid-precipitated material from the main compartment of the incubation flask was recovered by filtration, washed repeatedly, dried, and weighed. This fraction contained protein plus a small amount of TCA-insoluble carbohydrate and lipid. A weighed portion was combusted with the Van Slyke-Folch wet oxidation mixture and prepared for assay as barium carbonate *(5)*.

The procedure in each of the trials was the same up to this point. Treatment of the trichloroacetic acid filtrate varied from one trial to another; the procedure for Experiment 1 will be described and the modifications in subsequent experiments pointed out.

Experiment 1. Residual glucose was removed from the trichloroacetic acid filtrate by treatment with copper sulfate solution and calcium hydroxide followed by centrifugation. The alkaline supernatant was ether-extracted for 24 hours on a continuous Kutscher-Steudel extractor to remove alkaline ether-soluble substances. After adjustment of the pH to 1.5, it was extracted again with ether continuously for 24 hours. Aliquots of the alkaline ether extract, the acid ether extract, and the residual solution after ether extraction were evaporated by dryness, combusted, and radioassayed as barium carbonate. The acid ether extract should have consisted principally of lactic acid.

Experiment 2. In the second experiment it was found that all spermatozoa were immotile after the two washings to remove seminal plasma. It was decided to proceed with the experiment to determine if differences could be observed between immotile spermatozoa and those inactivated by the addition of sulfuric acid. The procedure described for Experiment 1 was followed. In addition, the preparations which were very active when assayed for radioactivity were examined by ion exchange chromatography. The columns were Dowex 1-formate, prepared and operated by the gradient elution method with continuously increasing concentrations of formic acid *(4)*. Two-ml. fractions were collected and an aliquot of each fraction was dried and assayed for radioactivity.

Experiment 3. The third experiment was similar in design to the first two, but steps were taken to determine the radioactivity in volatile substances. The copper sulfate–calcium hydroxide treatment of the trichloroacetic acid filtrate was omitted, since this treatment is known to remove compounds in addition to glucose. The trichloroacetic acid filtrate was adjusted to pH 9, unlabeled ethanol and acetate were added as carriers, and the solution was distilled until one-half the original volume had distilled over. This distillate, containing ethanol, was used for the preparation of ethanol derivatives (the 3,5-dinitrobenzoate and iodoform). The residual solution from distillation was adjusted to pH 1 and steam distilled. The distillate, containing acetate, was neutralized with barium hydroxide, evaporated to about 2 ml., and barium acetate was isolated and recrystallized from alcohol. The residual solution from the steam distillation was adjusted to pH 9, continuously extracted with ether for 24 hours, readjusted to pH 1.5, and again extracted with ether for 24 hours. Aliquots of each fraction were oxidized and radio-assayed as barium carbonate as in previous trials. As in Experiment 2, ion exchange chromatography was employed to check on the homogeneity of the more active preparations.

RESULTS AND DISCUSSION

The distribution of C^{14} in washed bovine spermatozoa exposed to uniformly labeled glucose-C^{14} in Ringer-phosphate solution is given in Table 1. Most of the activity appeared in the acid ether extract. Ion exchange chromatography indicated that lactic acid accounted for more than 90% of the activity in this extract. In motile spermatozoa the acid ether extract accounted for 94% of the recovered activity; however, appreciable activity did occur in other fractions. The total activity appearing in carbon dioxide was not large, but the specific activities in Table 2 show that the carbon dioxide formed was very active. This indicates that very little "carbon dioxide reserve" existed in the system and therefore dilution of the formed labeled carbon dioxide by unlabeled carbon was small.

The amount of total activity appearing in the trichloroacetic acid-insolubles is misleading and can be accounted for by the large quantity of carbon in this fraction. Specific activity of this fraction was very low, and even these values may be above the true values, as the large amount of carbon made adequate

TABLE 1

Distribution of C^{14} in bovine spermatozoa incubated for 3 hours with glucose-C^{14}

Fraction	Inactivated spermatozoa (Av. of Expt. 1 & 2)	Immotile spermatozoa (Expt. 2)	Motile spermatozoa (Expt. 1)
		(counts per min.)	
CO_2	298	5,134	3,050
Trichloroacetic acid-insoluble	6,238	1,275	33,600
Alkaline ether extract	1,522	2,800	1,450
Acid ether extract	8,905	211,800	994,050
Residue	3,778	6,008	28,205

TABLE 2

Specific activities of barium carbonate prepared from fractions of bovine spermatozoa incubated with glucose-C^{14}

Fraction	Inactivated spermatozoa (Av. of Expt. 1 & 2)	Immotile spermatozoa (Expt. 2)	Motile spermatozoa (Expt. 1)
	(counts per min. per μM C)		
CO_2	47	527	250
Trichloroacetic acid-insoluble	4	5	16
Alkaline ether extract	5	4	3
Acid ether extract	6	129	357
Residue	23	63	106

washing difficult. Specific activity of the trichloroacetic acid-insolubles was essentially negligible, indicating that protein and trichloroacetic acid-insoluble lipids and polysaccharides are not synthesized by spermatozoa from a glucose substrate under anaerobic conditions.

Ion exchange chromatography of the residue after acid ether extraction showed that about 50% of the activity present in this residual solution was due to lactic acid which had not been removed from the solution by ether extraction. A trace of activity was due to residual glucose; the remainder was found in the first few milliliters eluted from the ion exchange column and has not been identified. This same unidentified compound contributed about 5% of total activity present in the acid ether extract.

A comparison of motile and immotile spermatozoa shows that incorporation of C^{14} into lactic acid is related to the activity of the spermatozoa, whereas the incorporation of C^{14} into carbon dioxide and possibly into compounds in the alkaline ether extract was greater in the immotile spermatozoa. This is in agreement with the recent work of Koefoed-Johnsen and Mann *(8)*, who reported that cellular disorganization of spermatozoa resulted in an increase in oxygen uptake.

The specific activities of the fractions prepared in Experiment 3 are presented in Table 3. The 3-hour values presented for carbon dioxide and acid ether extract are in close agreement with those obtained for corresponding preparations in the trials presented in Table 2. Volatile compounds were recovered in this ex-

TABLE 3

Specific activities of barium carbonate preparations from Experiment 3

Fraction	Incubation for	
	1 hr.	3 hr.
	(counts per min. per μM C)	
CO_2	125	225
Trichloroacetic acid-insoluble	1	3
Neutral distillate	0	0
Acid stem distillate	43	32
Alkaline ether extract	20	11
Acid ether extract	258	347
Residue	1,001	540

periment, whereas no attempt was made to recover them in Experiments 1 and 2. The addition of unlabeled acetate and recovery by steam distillation, followed by isolation of the barium salt, indicates that acetate is a catabolite of glucose metabolism by bovine spermatozoa. However, neither the iodoform nor the 3,5-dinitrobenzoate derivatives of alcohol contained radioactivity.

The two principal catabolites, lactic acid and carbon dioxide, both had greater specific activity values after 3 hours than after 1 hour of incubation. Colorimetric determinations of lactic acid *(2)* were made after 0, 1, and 3 hours of incubation. The lactic acid present initially accounted for 29% of that present at 1 hour and 14.5% of that present at 3 hours. If corrections are made for this initial pool of unlabeled lactate, the specific activities for the newly formed lactate are 364 and 385 counts per minute per micromole of carbon for 1 and 3 hours, respectively. Thus, it appears that there is no appreciable difference between the specific activity of the lactic acid formed in 1 hour of incubation and that formed in 3 hours.

Since the glucose was uniformly labeled, any catabolite derived directly and exclusively from glucose should have the same specific activity as the glucose, subject to dilution by the endogenous reserve of that catabolite. It is doubtful that the washed spermatozoa contained sufficient reserve carbon dioxide to account for the difference in the specific activities from carbon dioxide at 1 and 3 hours. If, however, the carbon dioxide was derived from lactic acid rather than glucose, a greater specific activity of carbon dioxide at 3 hours than at 1 hour would be expected as a result of the initial reserve of lactic acid.

The large quantity of lactic acid derived from glucose, compared to the quantity of other catabolites, indicates that alternate pathways, if they exist in this system, contribute very little under the conditions of this study. This preponderance of lactic acid, considered in conjunction with the relationships among the specific activities, is regarded as evidence supporting the postulation that 2 *M* of lactic acid are formed per mole of glucose used and that the lactic acid is then metabolized to an extent dependent upon conditions of the experiment and the activity of the spermatozoa.

With the spermatozoa immotilized by washing, there is an indication that the metabolic pathway has been altered. If carbon dioxide was derived from lactic acid, the specific activity of carbon dioxide as well as lactic acid should be low in immotile spermatozoa. Since in this case the specific activity of the carbon dioxide greatly exceeded that of lactic acid, it appears that carbon must have been transferred from glucose to carbon dioxide without passing through lactic acid.

SUMMARY

Washed bovine spermatozoa were incubated with uniformly labeled glucose-C^{14} in Ringer-phosphate solution, and the products of catabolism were studied. The products were separated into classes by subjecting the incubation medium successively to absorption of carbon dioxide, precipitation with trichloroacetic acid, neutral distillation, acid steam distillation, alkaline ether extraction, and acid

ether extraction. The preparations showing considerable radioactivity were further purified by the preparation of derivatives or by ion exchange chromatography.

The major portion of the radioactivity appeared in lactic acid, although appreciable activity also was found in carbon dioxide, volatile acids and an unidentified compound. Relationships among the specific activities of the compounds studied indicate that spermatozoa probably metabolize glucose to lactic acid, and that the lactic acid may be broken down further to carbon dioxide, acetate, and other compounds. Results with immotile spermatozoa, however, indicated that carbon dioxide may be formed without passing through the intermediate stage of lactic acid.

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COMPLEMENTARY MILK AND ITS RELATIONSHIP TO LACTATION[1,2]

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Since Ott and Scott *(18)* reported that the injection of the posterior pituitary extract into the vein of the goat rapidly and greatly increased the secretion of milk, many workers *(8, 19, 26)* have experimented with the posterior pituitary extract and verified the results with different animals. The active factor of the extract was later found by Ely and Petersen *(5)* to be oxytocin.

Gaines *(6)* found that the injection of pituitary extract into goats milked "dry" never failed to yield more milk. Since then other workers have found similar conditions in cows and human subjects *(1, 2, 12, 13, 17, 24, 27)*.

Knodt and Petersen *(14)* postulated that the amount of both milk and milk fat produced may be dependent upon the completeness of the evacuation of the gland at each milking and that the failure of the cow to completely let down her milk may account for the rapid decline in milk production with the advance in lactation. Donker *et al. (3)*, using identical twin cows, showed that the milk let-down was a factor related to persistency, thus bearing out the theory set forth by Knodt and Petersen.

EXPERIMENTAL PROCEDURE

The main purpose of this study was to determine the amount of complementary milk and its relationship to other factors related to milk production.

However, before that could be done, it was found necessary to conduct preliminary experiments to solve the following problems: *(a)* method of ascertaining response, *(b)* method of administering oxytocin, *(c)* amount of time to allow between intravenous injection of oxytocin and removing complementary milk, and *(d)* dosage level of oxytocin to administer.

The results of the preliminary experiments showed that to determine the amount of complementary milk available a cow should first be milked out completely (hand-stripped if machine milked) and then the oxytocin should be administered before hand-stripping for the complementary milk.

It was also found that when the routes other than intravenous (intravaginal, intramuscular, and subcutaneous) were used, very high dosage and longer waiting time were necessary before response comparable to that of ten units of oxytocin administered intravenously was attained. It was concluded that for any routine survey of complementary milk, oxytocin should be administered intravenously.

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The results of the preliminary investigations showed that there was no significant difference in the amount of complementary milk obtained when the time between injection and hand-stripping was varied from 1 to 30 minutes.

When the dosage level was varied from 1 to 20 units of oxytocin administered intravenously, it was found that the response to one unit resulted in response equal to about 80% of the response to five units. Although the differences between the response to five units of oxytocin and ten units were not significant, the response to ten units tended to be greater than that to five units. The response to 20 units was not greater than the response to ten units. It is concluded, therefore, that ten units of oxytocin injected intravenously should be sufficient for the maximum response when the response is measured in terms of the completeness of obtaining the complementary milk.

On the basis of the results obtained in the preliminary experiments, the following procedure was used in determining the amount of complementary milk for the subsequent analysis. The cows were first milked normally under routine condition by machine. Then they were hand-stripped for the milk that was left, and ten units of oxytocin was administered intravenously. One to 10 minutes after the injection, the cows were again hand milked for the complementary milk.

All available lactating dairy cows in the University of Minnesota herd at St. Paul were tested for complementary milk about once a week for the period of one year. The hormone used to obtain complementary milk was the highly purified substance obtained mainly from the porcine pituitary gland containing 60 units of oxytocin per milliliter and will be referred to as oxytocin.

The butterfat percentage was determined by the Babcock method.

The method developed by Ludwick and Petersen *(15)* was used to determine the persistency of lactation of the animals involved. All calculations were based on a 305-day lactation period. The first 48 days were excluded; the remainder was divided into four equal parts, and the formula proposed by Ludwick and Petersen was employed to obtain the index of persistency.

The relationships between various factors related to milk production were determined by statistical analysis.

RESULTS AND DISCUSSION

As used here, complementary milk is the amount of milk in pounds obtained by oxytocin following a normal milking. Per cent complementary milk denotes the proportion of the total milking represented by complementary milk.

The results of 1,327 trials involving 62 cows (Table 1) show that the amount of complementary milk obtained ranged from 0.1 to 13.3 lb. with a mean of 1.7 lb. The per cent complementary milk ranged from 1.2 to 70.9% with a mean of 11.3%.

In Table 2 it will be noted that the amount of complementary milk of individuals ranged from 0.7 to 6.1 lb. with a mean of 1.8 lb. The per cent complementary milk of individuals ranged from 5.6 to 40.9 with a mean of 12.2.

The record of milk weights showed that the first milking after the injection of oxytocin and the removal of complementary milk was less than normal. The

TABLE 1
Summary of results of 1,327 trials involving 62 cows

Complementary milk	Mean	Range	
		Low	High
Pounds	1.7	0.1	13.3
Per cent	11.3	1.2	70.9

TABLE 2
Mean values of 62 individual cows

Complementary milk	Mean	Range	
		Low	High
Pounds	1.8	0.7	6.1
Per cent	12.2	5.6	40.9

question was whether the first milking was lower than expected only by the amount removed in determining the amount of complementary milk or whether the injection of oxytocin stimulated or inhibited milk secretion. To ascertain the effect of removing complementary milk, the amount removed by hand-stripping plus the complementary milk was added to the amount obtained at the first milking after the experimental milking. That amount was compared with the mean of the milk yield of the milkings immediately before and immediately after the one in question. The results of 220 trials involving 45 cows showed that the amount expected was 11.8 lb. and the amount obtained at the first milking, plus the complementary milk plus the amount removed by hand-stripping, was 12.1 lb. The difference of 0.3 lb. was not significant, which indicated that the use of oxytocin to remove complementary milk had no significant effect on the amount of milk produced. This is in agreement with the work of Schafer *(19)*, Hill and Simpson *(10)*, Shaw *(20)*, and others, who found that the total milk production was not significantly affected by oxytocin. However, Turner and Slaughter *(26)* reported that the reduction in milk removed at the subsequent milking was greater than the amount that could be accounted for by the milk removed with the aid of pituitrin. They concluded that pituitrin inhibited milk secretion. Smith *(21)*, Sprain *et al. (22, 23)*, and Donker *et al. (3, 4)* found that extensive use of oxytocin increased milk production rather than inhibiting it.

The per cent butterfat of the complementary milk ranged from 6.1 to 22.5 with the mean of 13.2. This is in very close agreement with the results of Dodd and Foot *(2)*, who reported that on the average the complementary milk contained no less than 13.42% butterfat. The correlation coefficient between per cent butterfat in normal milk and per cent butterfat in complementary milk was 0.31. The correlation coefficient between per cent butterfat in complementary milk and per cent complementary milk was 0.21. Both of the coefficients were significant at the 1% level, which means that the relationships, though not very high, were definite.

The correlation coefficient between the stage of lactation and pounds of complementary milk was –0.43 (significant at 1% level) as compared with the corre-

lation coefficient between the stage of lactation and per cent complementary milk, which was 0.04 (not significant). Thus, it is seen that the amount of complementary milk decreases with the progress of lactation, which is in agreement with the work of Swanson and Hinton *(24)* and Johansson *(13)*, who found that the amount of complementary milk decreased with the advancing lactation. However, it was found that the per cent complementary milk was not related to the stage of lactation and was relatively constant throughout the lactation.

In general the greater the level of production, the higher the amount of complementary milk. When the amount of production was correlated with the amount of complementary milk, the correlation coefficient was 0.53. The positive correlation is in agreement with Swanson and Hinton and Johansson, who reported that there was a direct relationship between the yield at normal milking and the amount of complementary milk. Turner *(27)* found direct relationship between the amount of complementary milk and total yield.

In this study it was also found that the correlation coefficient between the yield at normal milking and the per cent complementary milk was –0.02, which shows that there is no relationship between the amount of milk obtained at a normal milking and per cent complementary milk.

In view of the findings stated above, it seems fairly safe to state that the decrease in the amount of complementary milk with the progress in lactation is related to the stage of lactation only to the extent that the decrease in the amount of milk produced is related to the stage of lactation.

Some difference from the above finding was noted in one set of identical twin cows which were unable to let down their milk normally. During complete lactations one member of the set was injected with ten units of oxytocin every 3 days and the other every 6 days after normal milkings to determine the amount of complementary milk. The mean per cent of complementary milk for one member of the set was 34.3 and for the other, 35.3 (no significant difference). The correlation coefficient between the stage of lactation and the amount of complementary milk for the twins was –0.55, which was not significantly different from the mean of the herd, but the correlation coefficient between the stage of lactation and the per cent of complementary milk was 0.35, which was significant at 1% level. The significant increase, though not of great magnitude, in the per cent complementary milk as the lactation progressed indicated that the animals which were very poor normally in letting down their milk became worse as the lactation progressed.

It has been shown that, with the exception of the rare cases in which the animals were actually unable to let down their milk normally, there was no detectable relationship between the stage of lactation and per cent complementary milk. It was also shown that the amount of complementary milk was negatively correlated with the stage of lactation to the extent that the total production decreased with advance in lactation. Therefore, when dealing with complementary milk it must be realized that the absolute amount of complementary milk available will depend on the production level, whereas the per cent complementary milk is independent of the production level and the stage of lactation.

It is postulated at this point that the per cent of complementary milk is the factor that is inherited. Since it is independent of other factors, it is the characteristic that should be used in investigating the nature and the effect of complementary milk on factors influencing milk production.

To determine the relationship between the age of the animal and per cent complementary milk, a correlation coefficient was calculated on 46 cows whose age ranged from 23 to 85 months at parturition for the lactations concerned in this study. The coefficient of correlation was 0.18, which was not significant. Thus, it seems that the per cent complementary milk is relatively consistent throughout the life of the animal, at least throughout a greater part of its life. This is not in agreement with Johansson *(13)*, who stated that the averages of the different groups and sub-groups seem to indicate lower absolute and relative amounts of complementary milk for the young cows in their first lactation than for the older cows.

The index of persistency for the 46 animals with complete lactations ranged from 0.001 to 0.972. The relationship between the persistency of these animals and various factors will be discussed.

The correlation coefficient between the age of the animals (ranging from 23-85 months) and the persistency of lactation was –0.04, which indicates that there is no relationship between the age of the animal and persistency. This is not in agreement with the other workers who generally agreed that persistency decreased with age. Gaines *(7)*, however, stated further that persistency and initial rate studied indicated that persistency was independent of age, except as the initial rate of yield is related to age. Mahadevan *(16)* stated that after the drop from first to second lactation there was a steady increase in persistency with age. The discrepancy in results is probably due to different methods used in calculation, number of animals involved in the respective studies, and the presence or absence of unidentified environmental factors influencing the results. The lack of definite correlation in this study indicates the probable absence of the relationship between the factors considered.

The peak of production was considered as the average daily yield of the week having the highest production. In 45 cows the peak production ranged from 24.3 to 75.8 lb. of milk per day. The correlation coefficient between peak production and persistency was –0.004, which indicated that there was no significant relationship between the two factors considered. This is not in agreement with other workers who have reported that there was a negative correlation between initial rate and persistency of lactation. The disagreement in results is probably due to the method used in calculating the peak of production, the number of animals involved in respective studies, or to the relative production level of the animals in various studies. Since the higher the peak production, the greater the rate of fall must be for the animal to dry up in a given time, the high producer will naturally have greater negative correlation with persistency because of the method used in calculating this value.

The time of peak production was considered as the week of highest yield. The time of peak production for the 45 cows in this study ranged from the first to

the 11th week with 20 cows reaching the peak on the 5th week. The correlation coefficient between week of peak production and persistency was 0.50. This significant relationship indicates that the animals with poor persistency reach the peak of production early in the lactation and fall off more rapidly.

The index of the rise in butterfat percentage was determined by modifying the method developed by Ludwick and Petersen *(15)* for measuring persistency. All calculations were based on butterfat percentages of a 305-day lactation period. The first 48 days were dropped and the remainder was divided into four equal parts and treated as in Ludwick-Petersen formula for persistency except that no correction was made toward a hypothetical curve. The index was based on the deviation from the straight line. Thus, less than one indicates the decreasing butterfat percentage and the value over one indicates the increasing butterfat percentage with progressing lactation. The index was determined on 37 animals, and it was found that with the exception of three animals the trend of butterfat percentage was a gradual rise as the lactation progressed when the first 48 days were omitted. The index ranged from 0.9292 to 1.1384. The gradual rise in butterfat percentage with the progressing lactation is in agreement with Turner *(25)*, Jacobsen and Wallis *(11)*, Gould and Stout *(9)*, and others, who generally agreed that after a slight drop at the beginning of a lactation the per cent butterfat increased gradually to the end of the lactation.

The correlation coefficient between persistency and the trend of butterfat percentage was 0.20, which was not significant. Therefore, it is seen that persistency of lactation and the trend of butterfat percentage vary independently.

As stated earlier, the mean per cent complementary milk for the individuals ranged from 5.6 to 40.9% and the persistency values ranged from 0.001 to 0.972. The correlation coefficient between the two factors was –0.78, which was very highly significant. This means that as the per cent complementary milk increased, the persistency of lactation decreased. This bears out the theory set forth by Knodt and Petersen *(14)*, in which they stated that the amount of milk produced may be dependent upon the completeness of the evacuation of the gland at each milking and that the failure of the cow to completely let down her milk may account for the rapid decline in milk production with the advance in lactation.

The reason for the high inverse relationship between per cent complementary milk and persistency is somewhat speculative. Earlier development of intra-alveolar pressure because of a partially filled gland will account in part for the decrease in milk production, and perhaps other factors enter to produce a disuse atrophy of the mammary secretory epithelium. Thus, it seems that the per cent complementary milk left in the udder is one of the important factors influencing persistency of lactation.

Whether the per cent complementary milk is heritable and, if so, to what degree could not be ascertained by this study. Yet, there was a good indication that the condition is heritable because the members of identical twins tended to respond similarly.

SUMMARY AND CONCLUSIONS

In this study 62 cows were used in 1,327 trials over a period of 1 year to determine the amount of complementary milk available from individuals and to ascertain its relationship to other factors related to milk production. In addition, preliminary experiments were first carried out to determine: *(a)* the best method of ascertaining response, *(b)* the method of administering oxytocin effectively, *(c)* the amount of time to allow between intravenous injection of oxytocin and removing complementary milk, and *(d)* the dosage level of oxytocin to use.

On the basis of the preliminary experiments, it was found that to determine the amount of complementary milk the cows should first be milked "dry" (if machine milked they should be hand-stripped). Next, ten units of oxytocin should be administered intravenously followed by hand-stripping for complementary milk.

By means of the technique just stated, it was found that the amount of complementary milk varied considerably between 0.1 and 13.3 lb. per milking, with a mean of 1.7 lb. The per cent complementary milk ranged from 1.2 to 70.9 with a mean of 11.3. On the basis of individuals, the amount of complementary milk ranged from 0.7 to 6.1 lb. with a mean of 1.8 lb. The per cent complementary milk of individuals ranged from 5.5 to 40.9 with a mean of 12.2.

The butterfat percentage of complementary milk ranged from 6.1 to 22.5 with a mean of 13.2. There were significant positive correlations between butterfat percentage of complementary milk and butterfat percentage of normal milk and between butterfat percentage of complementary milk and per cent complementary milk, although the magnitude of the coefficient of correlation was low.

With the aid of statistical analysis wherever necessary the following conclusions were drawn from this study:

1. The use of oxytocin weekly to remove complementary milk had no detectable effect on milk production.

2. The amount of complementary milk decreased with progressing lactation and decreasing amount of yield at normal milking.

3. The per cent complementary milk was independent of the stage of lactation, the age of the animal, and the amount of yield at normal milking.

4. The per cent complementary milk was the better indication of the animals' response to milking procedure because it was relatively consistent throughout the lactations, whereas the absolute amount of complementary milk was influenced by the total amount of milk in the udder at the time of milking.

5. No relationship was found between persistency of lactation and the age of the animal, the peak of production, or the trend of butterfat percentage.

6. The animals which reached the peak of production earlier in lactation had poorer persistency of lactation.

7. There was decided negative relationship between persistency and per cent complementary milk.

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STUDIES ON THE PRODUCTIVE VALUE OF ROUGHAGES AND CONCENTRATES FOR LACTATION

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Several workers have observed that when dairy cows are fed an all-roughage ration following parturition, milk production declines steadily as the body fat is depleted and then becomes relatively constant at a level which can be maintained by the roughage consumed. At this time, replacing part of the hay with concentrates supplying an estimated equivalent amount of total digestible nutrients (TDN) results in increased milk production. Huffman and Duncan *(7, 9, 10, 11, 12)* observing this phenomenon, have interpreted it as evidence that concentrates contain a specific unidentified factor(s) which stimulates lactation. They suggest that prolonged feeding of hay alone has depleted the reserves of this factor(s), which had been stored in the cow's body during previous grain feeding. The inclusion of concentrates in the ration at this time, it is postulated, supplies the factor(s) and gives the cow the capability for increased milk production. Since the feeding of purified starch or sugar, instead of corn grain, did not increase milk yield, it was thought that energy alone was ineffective. More recently Burroughs *et al. (3)* have shown that starch lowers the digestibility of the ration, and several workers have shown a similar effect from high intakes of sugar or molasses. The Michigan workers have reported *(8, 14)* that certain hays contain varying amounts of the factor(s), early-cut hay being richer than mature hay. Corn silage containing the ears is a rich source of the factor(s) *(13)*, as are concentrates.

Earlier studies have shown the greater value of grains. Beach *(1)* found that digestible nutrients from corn were more useful for maintenance and for milk production than digestible nutrients from roughage. Moore *et al. (17)*, Sarrinen *et al. (19)*, and Irvin *et al. (15)* have attributed the observed increase in milk production to an increase in estimated net energy intake on rations containing concentrates. They have presented data from which they conclude that net energy alone can account for all of the increased milk production observed under these conditions. Recently Teichman *et al. (21)* observed greater increases in milk yield when corn grain replaced alfalfa hay on an equivalent TDN basis than on a calculated net energy basis.

A series of lactation experiments was conducted to obtain information on the comparative milk producing ability of roughages grown in New York and of rations balanced according to either Morrison's *(18)* TDN or estimated net energy (ENE) systems.

EXPERIMENTAL PROCEDURES AND RESULTS

Purebed cows from the Cornell dairy herds were fed an all roughage regimen 2 to 4 months after freshening according to the system reported by Huffman *(7)*.

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After depletion, the cows were fed various roughage and concentrate combinations in alternate periods in the initial tests. Later, reversal type tests were conducted with "depleted cows," in which 2-week periods of hay feeding were alternated with similar periods during which concentrates replaced part of the hay. Concentrates replaced hay in amounts calculated to supply the same amount of energy as either ENE or TDN as reported by Morrison *(18)*.

Milk production was recorded at each milking, and samples were taken from four consecutive milkings each week for fat tests. Body weights and feed intakes were recorded. Milk production is reported as fat-corrected milk (FCM) *(5)*.

Preliminary trial, 1948-49. Initial studies *(4)* were carried out in an attempt to confirm the observations of the Michigan researchers. It was soon found that cows depleted on hay increased in milk yield when fed equal intakes of TDN whenever a concentrate replaced part of the hay, thus substantiating the Michigan reports. The increases in milk yield appeared to be similar to those reported by Huffman and Duncan *(10)* and they occurred when TDN in hay was replaced with ground corn grain, corn distillers dried grains, corn distillers dried solubles, or a dairy concentrate mixture. The results were about the same whether alfalfa hay, a mixed clover-grass hay, or an early-cut timothy hay was fed as the basal ration.

Cows were fed also a basal ration of legume hay and well-eared corn silage following parturition. After approximately 3 months, when milk yields became fairly constant, part of the hay was replaced with an estimated equal amount of TDN from corn distillers dried grains or ground corn grain. The average daily yields of FCM of four Holstein cows when fed the hay-corn silage basal ration and for a 10-day period after concentrates replaced part of the hay on an equal TDN basis are shown in Figure 1. It is clear that the concentrates stimulated

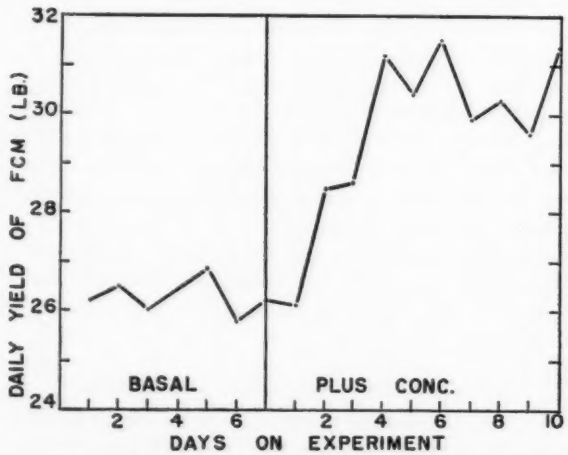

FIG. 1. Showing the influence on the yield of FCM from replacing hay with equal TDN from concentrates when the basal ration was hay and well-eared corn silage.

milk production even though the well-eared corn silage (40 lb. daily) was reported to be a good source of the hypothetical factor(s) *(13)*. On the basis of the lactation factor theory, this response would not be expected unless the basal ration was deficient in the specific factor(s) and unless the cows also were depleted, neither of which conditions would result on well-eared corn silage.

The results shown in Figure 1 can be explained most logically by assuming that the useful energy value of roughages is lower than estimated in digestion trials or that the energy value of concentrates is greater than so estimated. This assumption would explain the results of Huffman and Duncan and leave no need for the specific lactation factor theory. Morrison *(18)*, in summarizing feeding trial data for fattening cattle and for milk production, found that the TDN system overestimated the productive value of roughages when compared with concentrates. The difference was greatest for the poorer quality roughages. Because of this difference he prepared a table of estimated net energy (ENE) values in line with the observed usefulness of the various feeds in production experiments with No. 2 corn grain as the standard of comparison.

To test the view that useful energy from roughages and concentrates is the primary consideration involved in this lactation performance problem a series of trials was carried out to compare Morrison's *(18)* TDN and ENE values in replacing roughages with concentrates.

Double-reversal trials, 1950-51, 1951-52, 1952-53. Four double-reversal experiments were conducted with groups of cows fed roughages alone or roughages plus concentrates furnishing equal TDN or ENE, as outlined in Table 1. During 1950-51, the basal ration in one experiment contained mixed grass hay, corn

TABLE 1

The feeding plan of the double-reversal trials

Period[a]	Feeding system	
	Group A	Group B
1	Basal	Basal
2	Basal + conc. (TDN)[b]	Basal + conc. (ENE)[c]
3	Basal	Basal
4	Basal + conc. (ENE)	Basal + conc. (TDN)
5	Basal	Basal
6	Basal + conc. (TDN)	Basal + conc. (ENE)
7	Basal	Basal

[a] Periods 1 and 3, 3 and 5, and 5 and 7 were averaged for comparison with periods 2, 4, and 6, respectively.
[b] Ration balanced according to the total digestible nutrient system.
[c] Ration balanced according to the estimated net energy system.

silage, and sufficient soybean oil meal to supply adequate protein. The basal ration in the other experiments consisted of alfalfa hay or mixed alfalfa grass hay. With the systems of feeding used (Morrison's TDN vs. ENE, *18)* concentrates replaced about 20% more hay when substitutions were made on the basis of TDN compared to ENE.

The chemical composition of the feeds used in the four experiments is shown in Table 2. The average daily intake of feeds, the estimated intakes of TDN and

TABLE 2

Chemical composition of feeds used in lactation experiments

	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
			1950-51			
Alfalfa hay[a]	93.2	13.2	1.2	30.8	40.7	7.3
Mixed hay[b]	94.3	8.9	1.7	30.0	48.1	5.6
Corn silage	25.4	2.3	0.6	6.3	15.1	1.1
Soybean oil meal, solvent	90.5	47.1	0.6	6.0	30.2	6.6
Corn meal	87.7	8.2	2.4	2.2	73.4	1.5
Distillers dried rye grains	88.4	25.4	5.9	10.0	43.9	3.2
Distillers dried corn grains with solubles	86.5	25.9	7.7	8.8	38.6	5.5
			1951-52			
Alfalfa-timothy hay[c]	91.2	15.1	3.0	30.1	36.2	6.8
Corn meal	87.9	8.6	3.4	2.4	71.9	1.6
			1952-53			
Alfalfa-brome hay	92.0	16.1	3.0	25.6	40.6	6.7
Corn meal	87.2	8.5	3.2	2.3	71.8	1.4

[a] U. S. No. 2 alfalfa hay.
[b] U. S. No. 1 extra green mixed hay—except when musty, then sample grade.
[c] U. S. No. 2 alfalfa heavy timothy mixed hay.

ENE, and the FCM produced are summarized in Table 3. Statistical analyses of the production data were made by using a procedure derived by Henderson *(6)*.

In the 1950-51 trial, when alfalfa was used as the basal ration for six cows, statistically significant increases ($P < 0.05$) in milk production were observed when concentrates replaced part of the hay. Including grain in the ration according to TDN gave an average increase of 1.7 lb. additional FCM per day, which was a somewhat greater stimulation than the 1.3 lb. obtained when the ENE method was used. When mixed hay, corn silage, and soybean oil meal were fed as the basal ration for six cows, significantly more milk was produced when the rations were balanced according to TDN (an average increase of 3.5 lb. FCM per day) than when they were balanced according to ENE (an average increase of 0.8 lb. per day). Statistical appraisal of the two 1950-51 trials indicated that significantly more milk was produced ($P < 0.01$) when rations were balanced by the TDN than by the ENE method.

In the 1952-53 trial the animals consumed an average of 4.1 lb. more hay and produced an average of 3.0 lb. more FCM when fed rations calculated according to the TDN standard than when ENE was used. The extra hay consumed was more than sufficient to provide energy for the additional milk production. An average of only 0.5 lb. more FCM was produced when concentrates were included in the ration according to ENE compared with production on the basal ration. Likewise, in the 1951-52 trial the extra hay consumed could explain the extra milk produced on the TDN system.

Records of body weight changes were kept during each experimental period. All animals tended to increase in weight when hay made up the entire ration

TABLE 3

The average daily intake of feeds, TDN and ENE, and the yields of FCM on the different trials

No. cows	Date of trial	TDN system					ENE system				
		Hay	Grain	TDN	ENE	FCM	Hay	Grain	TDN	ENE	FCM
		(lb.)	*(lb.)*	*(lb.)*	*(therms)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(therms)*	*(lb.)*
6	1950-51	27.2	—	13.7	11.1	16.8	27.1	—	13.6	11.1	16.4
6	1950-51	12.5[a]	—	16.4	13.1	18.1	13.5[a]	—	16.8	13.7	20.8
5	1951-52	32.0	—	15.8	12.7	26.5	32.1	—	15.8	12.8	26.8
4	1952-53	34.3	—	17.0	13.6	27.2	34.6	—	17.2	13.7	27.0
21	Mean	26.5	—	15.7	12.6	22.2	26.8	—	15.8	12.8	22.8
6	1950-51	18.0	6.0	13.4	11.7	18.5	15.5	6.0	12.2	10.7	17.7
6	1950-51	3.1[a]	6.0	15.9	13.6	21.6	2.1[a]	6.0	15.4	13.3	21.6
5	1951-52	19.2	8.7	16.5	14.2	28.2	14.2	9.6	14.7	13.3	27.7
4	1952-53	25.1	6.7	17.8	15.3	30.5	21.0	6.7	15.7	13.7	27.5
21	Mean	16.4	6.8	15.9	13.7	24.7	13.2	7.1	14.5	12.8	23.6
Difference		–10.1	6.8	0.2	1.1	2.5	–13.6	7.1	–1.3	0.0	0.8

[a] Also fed 44 lb. of corn silage and 3.6 lb. of soybean oil meal daily.

and to decrease in weight when concentrates were fed, in agreement with the data of Huffman and Duncan *(13)*. It was not possible to differentiate between changes in the animals' tissues and fill or water retention in these studies.

DISCUSSION

The consistent increase in production when concentrates were used to replace part of the hay on an equal TDN basis is evidence that the TDN system does not accurately evaluate these two feed sources for milk production. An average of the data from all trials shows that the cows produced 2.5 lb. more FCM per head per day when 6.8 lb. of concentrates replaced 10.2 lb. hay containing almost equal TDN but more ENE. Replacing hay with concentrates according to the ENE system resulted in an average increase of 0.8 lb. FCM per head per day on less TDN but the same ENE intake. The significantly greater milk yield from the TDN above the ENE feeding system shows rather conclusively that Morrison's ENE values more accurately estimate the comparative productive usefulness of the energy in the roughages and concentrates studied than do the TDN values. The results suggest, however, that even the ENE system leaves something to be desired, in agreement with Smith *et al. (20)*. Clearly, the data do not support the lactation factor theory, since the extra milk resulted largely from feeding greater amounts of hays which were judged deficient in any factor(s) by the Michigan criterion. Furthermore, responses were about the same when the basal ration contained well-eared corn silage and, in one trial, soybean oil meal. The data presented here and published elsewhere *(4, 15, 17, 19, 21)*, we believe, support the view that the efficiency of utilization of energy of the various feeds is largely or entirely involved rather than an unidentified factor(s) which stimulates milk secretion. Undoubtedly, several poorly defined interrelationships among known feed nutrients are involved, such as bulk, mentioned by Huffman and Duncan *(12)*, and/or other variables described by Armsby and Forbes as influencing the net energy value of livestock feeds. It is also possible that unrecognized factors influence rumen function *(2)* and energy utilization, but such factors would not be specific for lactation. In the Michigan reports various recognized relationships have not been considered.

After parturition, the amount of milk produced is the result of an equilibrium among several factors, such as *(a)* the hormonal stimulation for production, *(b)* the nutrients available, and *(c)* limitations set by the cow's genetic ability for production. Our present knowledge gives us little control over items *a* and *c*. Nutrients available for milk production can come from two sources, daily feed and stores in the animal's body. Animals well managed at parturition usually have large body stores of energy as well as a high impulse for production. As long as body stores last and no disease is present, production will be limited by hormonal stimulation or genetic capacity (which may be the same). However, when body stores of energy become depleted, the daily intake of energy becomes a limiting factor in production. At this time the animal becomes very sensitive to fluctuations in the energy value of its feed.

Since most published evaluations of feeds, especially for TDN, have been made under conditions of maintenance, growth, or fattening, it is not surprising that these values are not accurate for lactation. Calculated net energy appears to fit this situation more closely than TDN, but the accuracy of all calculated values can be questioned.

As this manuscript was being prepared, the excellent report of Martin *et al. (16)* presented comparisons similar to ours between TDN and ENE. They conclude: "Neither TDN nor ENE could be said to be superior to the other as an indicator of the worth of a ration, though ENE did seem to be somewhat more consistent over a wide range of hay:concentrate ratios."

Clearly, more accurate methods of evaluating the productive usefulness of feeds for lactation are needed. Huffman and Duncan deserve much of the credit for showing this need. To obtain accurate estimates of the value of various feeds for lactation, tests must be made on lactating cows in which increases or decreases in body energy stores are accurately appraised. Such measurements are difficult, if not impossible, to evaluate with short test periods as used in these trials and the others cited.

SUMMARY

Reversal type experiments were conducted with 25 lactating cows to determine the effect of replacing part of a roughage ration with concentrates supplying equal amounts of TDN or ENE. TDN proved to be less accurate than ENE for comparing the energy value of roughages and concentrates for milk production. ENE appeared to slightly overrate the value of roughages in comparison to concentrates for lactation, but to a lesser degree than TDN.

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SOME CHEMICAL CHARACTERISTICS OF GRASS AND LEGUME SILAGE MADE WITH SODIUM METABISULFITE[1,2]

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The use of sodium metabisulfite as a preservative for grass silage was first suggested by Pennsylvania workers *(4)*, in 1952. A subsequent progress report by them *(5)*, in 1953, gave the results of trials carried out with this preservative and listed the benefits obtained by its use. In particular, the reduced nutrient losses from the silo and improved color and palatability of the final product suggested that fermentation in the ensiled forage was suppressed to a considerable extent.

Sodium metabisulfite is a known bactericide *(7)*, which has been used for many years in the wine industry and the preservation of dehydrated and fresh fruits. The present study was undertaken in order to determine the nature of the fermentation occurring in grass silage ensiled with the addition of sodium metabisulfite.

EXPERIMENTAL PROCEDURE

During 1953 about 50 tower silos on farms in Pennsylvania were filled with grass silage under the supervision of two of the authors.[4] Several unwilted grass and legume forages were ensiled, 19-26% dry matter, and sodium metabisulfite was applied as the dry powder, at the silage blower, at the rate of 8 to 10 lb. per ton of green forage. During the following winter, samples of the resulting silage were obtained from these silos, and extensive analyses were carried out to determine the nature of the fermentation which had occurred.

A water extract of the fresh silage was prepared in a Waring blendor and used for the following determinations. The pH was determined with a conventional glass electrode pH meter. Volatile acids were determined by the method of Wiegner as modified by Watson and Ferguson *(12)*. This technique makes it possible to report volatile acids separately as acetic or butyric. Propionic acid, if present, will give inaccurate values for acetic acid and butyric acid, but propionic acid does not commonly occur in silage. If zero values for butyric

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acid are obtained, as in two-thirds of the samples analyzed in this work, interference from propionic acid is ruled out. For use with silage extracts containing residual sulfite, the technique requires slight modification, since sulfurous acid will distill over and interfere with the titration. The addition of small amounts of potassium biiodate to the extract was found to be effective in this respect; other oxidizing reagents would probably serve as well.

Lactic acid was determined by an adaptation of the colorimetric procedure of Barker and Summerson *(2)* and reducing sugar by a combination of the clarification technique of Somogyi *(10)* and the colorimetric procedure of Benedict *(3)*. Titratable acidity, volatile bases, and amino acids were determined by the method of Foreman *(6)*. Total residual sulfite (free and combined) was determined directly on the fresh silage by the technique of Nichols and Reed *(8)*.

RESULTS AND DISCUSSION

For purposes of statistical analysis, the 48 samples of silage were divided into three groups on the basis of the botanical composition of the ensiled forage. These groups were: A, predominantly grass; B, predominantly legume; and C, mixed grass and legume. The mean values and standard deviation for each constituent determined for each of the three groups of silages and for the complete 48 samples are shown in Table 1.

Significant differences between the mean values were obtained for legume silages compared with grass and for the mixed grass compared with legume silages. Legume silages contained slightly greater amounts of acetic acid, lactic acid, and volatile bases and smaller amounts of reducing sugar. These results probably reflect the differences in composition of legumes as compared to grasses, in particular the higher protein content, lower sugar content, and higher buffering capacity. No significant differences were found between grass and mixed grass legume silages.

The differences reported, although significant in some cases, are relatively small compared to the gross differences between the chemical composition of these silages and the reported composition of silages made by a variety of other techniques. In the following discussion, therefore, reference will be made only to the mean values for the entire 48 samples.

The mean value of 28.4% for the dry matter content appears to be a little high for unwilted forage, but, since the latter loses water by seepage on ensiling, a corresponding rise in dry matter content of 3-10% is commonly observed. No deliberate wilting was used on the forages ensiled in these trials.

In this study consistently low values were obtained for lactic, acetic, and butyric acids. The values are approximately one-half the values reported by Watson and Ferguson *(12)*, Stone *et al. (11)*, and Archibald *(1)* for grass and legume silages made by various other techniques. It is therefore clear that sodium metabisulfite partly inhibits acid production by bacteria in silage. This inhibition represents a departure from the accepted principle of stimulating acid formation which underlies the majority of ensiling techniques currently in use.

TABLE 1

Fermentation products in grass, legume, and mixed grass legume silages made with sodium metabisulfite (mean values)

	A Grass	B Legume	C Grass and legume	All samples
Number of samples	17	15	16	48
Dry matter *(%)*	28.9 ± 4.6	27.7 ± 3.2	28.5 ± 2.8	28.4 ± 4.1
pH	4.50 ± 0.29	4.43 ± 0.25	4.54 ± 0.26	4.49 ± 0.26
Per cent on dry matter basis				
Acetic acid	1.12 ± 0.73	1.71 ± 0.46[a, b]	0.91 ± 0.50	1.24 ± 0.66
Butyric acid	0.08 ± 0.14	0.07 ± 0.18	0.12 ± 0.22	0.09 ± 0.18
Lactic acid	3.25 ± 3.00	4.95 ± 1.46[a]	2.91 ± 1.87	3.67 ± 2.36
Amino acids (calculated as crude protein)	4.52 ± 1.23	5.45 ± 1.40	5.11 ± 0.83	5.01 ± 1.22
Volatile bases (calculated as crude protein)	1.04 ± 0.40	1.42 ± 0.33[a, c]	0.91 ± 0.41	1.16 ± 0.37
Reducing sugar (as glucose)	4.63 ± 4.24	1.23 ± 0.65[a, b]	5.12 ± 2.50	3.97 ± 3.40
Total residual sulfite (as So_2)	0.266 ± 0.179	0.264 ± 0.144	0.347 ± 0.230	0.295 ± 0.192

[a] Difference B – C significant, $P < 0.01$.
[b] Difference B – A significant, $P < 0.05$.
[c] Difference B – A significant, $P < 0.01$.

The low values for butyric acid are in good agreement with the pleasant odor which is a prominent characteristic of bisulfite silage. Two-thirds of the samples examined contained no detectable amounts of butyric acid, and the remaining one-third contained amounts of the order of 0.5% of the dry matter, or less.

Protein breakdown in the silages was controlled to a satisfactory extent in comparison with other types of silage *(12)*. Amino acids were formed in considerable amounts, but as this type of breakdown by hydrolysis involves no loss of feeding value its magnitude is relatively unimportant. Volatile bases, comprising mainly ammonia and the volatile amines, averaged only 1.16% of the dry matter, calculated as crude protein. Breakdown of amino acids to simple volatile nitrogenous compounds was thus satisfactorily controlled.

The reducing sugar content of the silages showed wide variation, and the legume silages had a significantly lower sugar content (1.23%) compared to grass (4.63%) and grass-legume (5.12%). Legumes, particularly alfalfa, have lower sugar contents than do grasses, and this, together with the slightly greater acid production, is responsible for the differences observed. In general, values were higher than those reported by Archibald *(1)* for grasses and legumes ensiled without added carbohydrates. This fact is in agreement with the restricted nature of the fermentation shown to occur in bisulfite-treated silages.

It is widely accepted that a low pH of the order of 4.0 to 4.5 is one of the chief criteria of a well preserved silage. The pH of the ensiled forage is lowered to within the desired range by the production of organic acids by bacterial fermentation of carbohydrates already present in the forage or added during the process of ensiling. Direct acidification with mineral acids has also been used to achieve the same objective.

The mean pH value for all samples was 4.49, with no significant differences observed between the three groups. This mean value barely falls within the desired range, and, in fact, 17 samples fall in the range 4.6-5.0. The individual data showed a number of silages with pH values of 4.8 and 4.9 which were better preserved, as judged by the quantities of fermentation products formed, than silages with pH values within the accepted range. All samples of silage analyzed were well preserved, particularly with respect to protein.

Although sodium metabisulfite is an acid salt of sulfurous acid, its action cannot be explained in terms of its acid content. The addition of 8 lb. of sodium metabisulfite to a ton of green forage is equivalent to adding only 4.1 lb. of sulfuric acid, or only about one quarter of the amount of acid required for direct acidification procedures. Repeated observation on freshly ensiled forages to which sodium metabisulfite was added have shown pH values of the order of 5.3 to 5.5, confirming that only a limited amount of direct acidification is achieved.

It is therefore suggested that, as they are well preserved in the absence of considerable added or developed acidity, bisulfite silages form a new class of silages, characterized by the inhibiting effects of the HSO_3^- ion, rather than the H^+ ion, resulting in reduced acid formation and protein breakdown by bacteria.

Acidity, as measured by the pH values, does play a part in the action of bisulfite, however. The dissociation of sulfurous acid is controlled by the two equilibria given by Rahn *(9)*.

$$\frac{(H^+) \times (HSO_3^-)}{(H_2SO_3)} = 0.017 \text{ and } \frac{(H^+) \times (SO_3^{--})}{(HSO_3^-)} = 5 \times 10^{-8}$$

Increased hydrogen ion concentration results initially in a suppression of the dissociation of the HSO_3^- ion to H^+ and SO_3^{--} and finally in suppression of the dissociation of the H_2SO_3 molecule itself. Maximum concentration of HSO_3^- ions is obtained between pH 3-4, but at pH 5, 67% of the sulfurous acid present will be in the form of the HSO_3^- ion. Since, according to Rahn, the HSO_3^- ion is more toxic than the SO_3^{--} ion, a shift in pH value from 5 to 4 should result in more effective inhibition of bacterial action for the same initial amount of sodium metabisulfite added.

The total residual sulfite (both free and combined) in this series of samples was found to average 0.295% SO_2 in the dry matter, with a fairly wide variation about this mean. The mean value is equivalent to 840 p.p.m. in the fresh silage and represents only 25% of the amount of sodium metabisulfite originally added. The loss of 75% of the added bisulfite can be explained in several ways. Sodium metabisulfite is a powerful reducing agent when in solution and will oxidize to sodium bisulfate. Therefore, any factor tending to increase the access of air or reducible material to the bisulfite will correspondingly lower the residual sulfite in the silage. Such factors are the dry matter content of the ensiled forage, the rate of filling, type of silo, degree of consolidation, and type of seal used. There is also the mechanical loss of sulfite in seepage from ensiled unwilted material and the possibility of some loss by evolution of sulfur dioxide gas.

Since the data summarized in Table 1 provided evidence of the action of sodium metabisulfite in partially inhibiting acid formation, a relationship between total residual sulfite and the amount of acid formed seemed likely. Taking the sum calculated as milliequivalents of the amounts of acetic, butyric, and lactic acids formed, the present data showed an inverse correlation between this value and the concentration of total residual sulfite. The value for the correlation coefficient, r, was –0.465, which is significant, $P < 0.01$.

SUMMARY

Forty-eight samples of grass and legume silage made with sodium metabisulfite at the rate of 8-10 lb. per ton of green forage have been analyzed for the common fermentation products found in silage. The data show that the production of acetic and lactic acids by bacterial fermentation was limited and inversely correlated with the total residual sulfite concentration. Butyric acid production was almost eliminated. Protein breakdown was inhibited to a satisfactory degree, despite pH values higher than normal. The evidence suggests that the forages were preserved primarily by the action of the HSO_3^- ion, instead of the H^+ ion, as is usual in other conventional types of silage.

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THE EFFECT OF METHODS OF ADDING EGG YOLK AND MONOSACCHARIDES ON THE SURVIVAL OF FROZEN BULL SPERMATOZOA

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The technique of freezing semen is complicated by the fact that the extenders required for processing each sample of semen include both a nonglycerol and a glycerol fraction *(3, 5, 8, 9)*. It has been suggested by Dunn and Hafs *(4)* that the relative composition of the two fractions may influence the tolerance of spermatozoa to the freezing process as much as the composition of the final admixture.

The egg yolk–citrate extenders described by English workers *(7, 8, 9)* contain egg yolk in the nonglycerol medium only. On the other hand, Bratton *et al. (2)* and De Groot *(3)* added egg yolk to both fractions of the extender. The latter author also added fructose in both portions, whereas other workers *(1, 6)* have had better results with pentose sugars added in the glycerol portion.

This investigation was initiated to compare the effect of including 25% yolk in both the glycerol and nonglycerol fractions with the effect of including 50% yolk in the nonglycerol and none in the glycerol fraction on motility and fertility of bull spermatozoa thawed from –79° C. The effect of adding one of three monosaccharides either entirely in the nonglycerol fraction, equally in both fractions, or entirely in the glycerol fraction was laboratory tested in a factorial experiment.

EXPERIMENTAL METHODS

Laboratory trial. Soon after the semen samples were collected they were extended at 38° C. in the desired nonglycerol fraction to a concentration of 30 million total spermatozoa per milliliter. The partially extended semen was allowed to cool slowly to 5° C. Three and one-half hours after the initial dilution an equal volume of the glycerol fraction was added in five aliquots equally spaced over a half hour so that the dilution was complete 4 hours after the initial dilution. The sperm were allowed to equilibrate with the glycerol for 18 hours before freezing.

The semen containers used for freezing were Pyrex brand glass ampules. Freezing was accomplished at the rate of 0.8° C. per minute from 5° C. to –12° C., after which the remaining drop in temperature was accomplished as rapidly as possible by pouring powdered Dry Ice into the freezing bath.

Ampules were thawed in a 5° C. water bath, and the contents of three replicate ampules were composited in a single 3-in. cork-stoppered glass test tube for motility estimations. Duplicate motility estimations were made from each tube

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just after thawing and averaged for statistical analysis. The composite samples were stored at 5° C., and motility estimations were made again 24 and 48 hours after thawing. The person making the estimations was not allowed to know which treatment he was examining.

TABLE 1

Composition of the nonglycerol and glycerol fractions for the two methods of adding egg yolk

	Nonglycerol fraction		Glycerol fraction			Final extender		
	C[a]	Y[a]	C	Y	G[a]	C	Y	G
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Yolk added in nonglycerol fraction	1.47	50	2.53	0	14.0	2.00	25	7.0
Yolk split between both fractions	1.47	25	2.53	25	14.0	2.00	25	7.0

[a] C = sodium citrate dihydrate; Y = egg yolk; G = glycerol (volume per volume).

The two methods of adding egg yolk are shown in Table 1. The three monosaccharides (fructose, glucose, and xylose) were added to the extenders shown in Table 1 either entirely in the nonglycerol fraction, equally in the nonglycerol and glycerol fractions, or entirely in the glycerol fraction. Wherever sugar was present in the final extender it constituted 1% (weight per volume) of the final composition. Streptomycin was added to a concentration of 1,000γ per milliliter in the nonglycerol portion of all laboratory and field trial extenders so that the final concentration was 500γ per milliliter.

Field trial. The field trial consisted of a simple comparison of the two extenders shown in Table 1. Collections from seven Holstein bulls were split equally between the two extenders. The mechanics of dilution, glycerolation, equilibration, and freezing were the same as in the laboratory trial.

Eight inseminating technicians who were using frozen semen exclusively cooperated in the trial. These technicians were divided into two approximately equal groups as far as numbers of cows bred and technician proficiency were concerned. One technician group used one treatment, the other group the other treatment for 1 week, at which time the groups were switched with respect to treatment. It developed that, to obtain an adequate number of services, the process had to be repeated for most bulls. This means that semen of one collection was used over a period of 4 weeks, or up to 5 weeks after the date of collection. Semen was delivered to the technicians' kits in Dry Ice and isopropyl alcohol within 1 week after collection. The trial was conducted during April and May, 1954.

Nonreturn information was tabulated directly from the technicians' copies of the breeding receipts.

RESULTS AND DISCUSSION

Laboratory trial. The average motilities of the 20 treatments immediately after thawing are shown in Table 2. An analysis of variance with 259 degrees of freedom revealed highly significant differences ($P < 0.01$) between the two

TABLE 2

Effects of methods of adding yolk and sugars on the percentage of motile spermatozoa surviving freezing to −79° C. (av. of 13 ejaculates)

	Method of incorporating egg yolk					
	A[a]			AB[b]		
	Method of adding sugar			Method of adding sugar		
Sugar	A	AB	B[c]	A	AB	B
	(% motile)			*(% motile)*		
Fructose	19.2	26.5	26.1	27.9	32.2	35.8
Glucose	18.8	24.1	24.9	25.9	32.7	31.8
Xylose	15.3	23.4	21.8	22.5	31.9	30.2
None (control)		22.3			30.5	

[a] Added in nonglycerol fraction.
[b] Added equally in both fractions.
[c] Added in glycerol fraction.

methods of adding yolk, among the three methods of adding monosaccharides, and among the three monosaccharides. None of the interactions between or among the main effects approached statistical significance. The extremely large F ratio (111.64) calculated for the method of adding yolk permits little doubt of the superiority of the method of splitting the yolk equally between the two portions of the extender. Differences among the three sugars may be attributed primarily to the relatively poor results obtained with xylose. Xylose appeared to have little if any benefit when compared to the controls. Glucose and fructose reacted similarly, although fructose resulted in slightly higher motilities.

None of the three sugars reacted favorably when added entirely in the nonglycerol fraction. Sperm subjected to this treatment were exposed to a 2% solution of sugar for nearly 4 hours, after which time the concentration of sugar was halved. Little preference was exhibited between the two other methods of incorporating sugar.

It is worthy of mention that another treatment was run simultaneously with this experiment, although not an integral part of the factorial. The final egg yolk–citrate–glycerol concentration of this extender was the same as the extenders shown in Table 1, and egg yolk was added by splitting it equally between the nonglycerol and glycerol fractions. The difference was that the concentration of citrate in both the nonglycerol and the glycerol fraction was 2.00%. The average motility of 13 samples treated in this manner was 24.1%, considerably lower than the split yolk control shown in Table 2.

Motility studies made after storage at 5° C. for 48 hours showed relatively greater differences among treatments.

Field trial. The results of the fertility trial are shown in Table 3. The difference of 8.3% between the two treatments was highly significant statistically ($P < 0.01$) as tested by the analysis of variance.

TABLE 3

Fertility of semen extended by two methods of adding egg yolk

Bull	Method of incorporating yolk			
	Nonglycerol fraction		Both fractions	
	First services	60- to 90-day nonreturns	First services	60- to 90-day nonreturns
	(No.)	*(%)*	*(No.)*	*(%)*
1	142	66.7	164	67.1
2	159	52.2	133	61.6
3	81	63.0	96	71.9
4	36	58.3	35	68.6
5	74	63.5	70	75.7
6	122	52.4	108	64.8
7	128	64.8	167	70.6
Totals	742		773	
Averages		59.7		68.0

SUMMARY

The inclusion of 25% egg yolk in both the nonglycerol and the glycerol fractions of extenders for frozen semen was compared with including 50% egg yolk in the nonglycerol fraction and none in the glycerol fraction. The final compositions of these two extenders were identical. The average percentages of motile sperm immediately after thawing were 30.5 and 22.3 for the two methods, respectively, and the average 60- to 90-day nonreturn percentages for 1,515 first services were 68.0 and 59.7, respectively. These differences were highly significant statistically ($P < 0.01$).

Fructose, glucose, or xylose was added to give a final concentration of 1.0% by adding the sugar entirely in the nonglycerol fraction, equally in the nonglycerol and glycerol fractions, or entirely in the glycerol fraction. The three methods of incorporating the sugars resulted in 21.6, 28.4, and 28.4% motile sperm, respectively, immediately after thawing. The average percentages of motile sperm in the extenders containing the three sugars were: fructose, 27.9; glucose, 26.4; and xylose, 24.2. In both cases differences were highly significant ($P < 0.01$) when tested by means of the analysis of variance.

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PEOPLE and EVENTS
in the Dairy Science World

Chester Linwood Roadhouse

CHESTER LINWOOD ROADHOUSE, born on a dairy farm near Watsonville, Calif., Jan. 5, 1881, got his start in the dairy industry at the age of five by trying unsuccessfully to milk a cow. Soon after, the family leased the dairy and moved to Fresno County in the interior valley and developed a raisin-grape and fruit acreage. A dairy was operated in connection with this enterprise. In his high school years he worked during summer vacations with grain harvesting crews, and the long hours convinced him that there must be a better method of making a living.

C. L. Roadhouse

Roadhouse entered the Univ. of California as a regular student in agriculture in 1902. Courses in bacteriology and animal diseases were of special interest. After conferring with an instructor who was a graduate of Cornell Univ. and meeting DR. V. A. MOORE of the Cornell Veterinary faculty, he decided to continue his studies at that university. He was graduated with the D.V.M. degree in 1906. During his senior year he played left end on the varsity football team and in the spring semester won his bout in boxing in the middleweight class. Immediately after graduating he passed the federal Civil Service examination and was stationed in San Francisco as a veterinary inspector in the Bureau of Animal Industry, USDA.

In 1909, a group of doctors and interested citizens of San Francisco formed the Milk Improvement Assoc. and raised funds to begin the supervision of the city milk supply. The chief of the Bureau of Animal Industry recommended Roadhouse to inaugurate the work in connection with the Dept. of Public Health. He resigned from the federal position and accepted the appointment, furnishing his own transportation and out-of-town traveling expense for a few months in order to get the work started.

Previously, the dairies outside the city had not been supervised. Sanitary conditions and the bacterial quality of the milk, in general, were bad. After supervision for one year, the count in 70% of the milk samples from all sources averaged lower than 100,000 per milliliter. At that early date such bacterial quality was considered satisfactory for unpasteurized milk.

In 1910, Dr. Roadhouse accepted an appointment as city veterinarian for Berkeley, Calif., and started the supervision of the milk supply of that city. In 1911, he was appointed an instructor in veterinary science in the Univ. of California and manager of the Univ. Certified Dairy, teaching courses in sanitary milk production and common diseases of animals and doing extension work in these fields. In addition he was veterinarian and bacteriologist for the Medical Milk Commissions of San Francisco and Alameda Counties, supervising six dairies in northern California that were producing certified milk. At the 1915 Panama Pacific Exposition held in San Francisco he was one of the judges in the milk scoring contest, where approximately 450 samples from the United States and some from Canada and Hawaii were entered.

In 1917, after spending several months visiting dairy departments in eastern colleges and universities, Dr. Roadhouse became professor of dairy industry and chairman of the department in the Univ. of California at Davis. From the beginning of his connection with the department he was actively engaged in instruction, research, and administration. A new dairy industry building was constructed in 1922, and the teaching and research staff, including assistants in the manufacturing laboratories, had increased to 19 members by 1923.

Illness in 1945 made it necessary for him to relinquish his administrative duties, but after an absence of several months he returned to carry on an active teaching program. In 1951, he retired after 40 years of service, but he has continued as professor emeritus to be available for consulting work. Members of industrial firms and former students confer often with Dr. Roadhouse in his Davis home, where he has now moved his office. His role as a teacher and counselor is particularly noteworthy. Some of his former students are among the leaders in the California dairy industry, several having achieved national recognition. He is presently devoting considerable time to writing.

Dr. Roadhouse has been unusually active in dairy associations. He was president of A.D.S.A. in 1935 and served on the editorial board of the *Journal of Dairy Science* from 1917 to 1926. He was president of the Pacific Slope Dairy Assoc. from 1922 to 1946 and president of the Intern. Assoc. of Milk Sanitarians in 1921. He had the distinction of being an official U. S. delegate to the Intern. Dairy Congress in Ber-

lin in 1937. He spent a sabbatical year at the Swiss Dairy Research Station at Liebefeld, near Berne, Switzerland, studying the fundamentals of the flavors of milk. He and DR. G. A. KOESTLER at that time stated that the chlorides and lactose of milk are more concerned with the primary taste than are the other normal constituents of milk. He was an active member of the Institute of Food Technologists, the Calif. Academy of Science, A.D.S.A., Sigma Xi, Alpha Zeta, and Theta Delta Chi.

Some 130 published articles appear in scientific and popular literature under Dr. Roadhouse's authorship or co-authorship. These papers deal with dairy technology, flavors, and chemistry of milk. In collaboration with DR. J. L. HENDERSON, he wrote a widely used text, *The Market Milk Industry*, recently revised.

He has been very active in civic improvement and has contributed in many ways to the growth of the community. He is a charter member of the Davis Rotary Club and has recently written a history of the local organization. He was chairman of the board of trustees when the local Community Church was built and president of the Davis Chamber of Commerce in 1951.

In 1952, Dr. Roadhouse received the A.D.S.A. Honorary Member Award. He is also an honorary member of the California Dairy Industries Assoc. and has continued as an advisory member of the Calif. Dairy Council.

Dr. Roadhouse had expected to travel extensively after his retirement, but the prolonged illness of Mrs. Roadhouse interfered with the plans. In addition to other activities, gardening is one of his hobbies, but he always has time to enjoy the visits of friends and former students.

C. A. PHILLIPS

East Lansing Meeting Well Attended

A total of 1,361 were registered for the annual meeting held at East Lansing, June 20-23. Of this number 842 were men, 316 were women, and 203 were children. The facilities were excellent and the host group is to be congratulated for the efficient manner in which the details of the meeting were planned and executed. Even the weather was under control.

Representatives were present from 48 states, Washington, D. C., Canada, and eight other foreign countries. Our group is no longer a national association but has become international in scope. Possibly the name should be changed to make it better correspond to the scope of our activities and membership.

Heard at the Annual Meeting

O. E. REED, director of Livestock Agricultural Research Service, USDA, will retire Sept. 1, 1955.

N. R. BAYLEY, associate professor in the Dairy Department at the Univ. of Minnesota, will become assistant head of the Breeding, Feeding, and Management Section, Dairy Husbandry Research Branch, USDA, August 1.

C. D. DAHLE, professor of dairy manufactures at Penn. State Univ., will retire from active duty August 1. Mr. and Mrs. Dahle are planning to travel in Europe this fall.

R. F. HOLLAND, professor of dairy industry at Cornell Univ., succeeded J. M. SHERMAN as head of the Department July 1.

O. F. HUNZIKER and E. S. GUTHRIE, charter members of the association formed on the Illinois campus in 1906 which later became the A.D.S.A., were among those attending the 50th meeting.

The schedule for future meetings of A.D.S.A. is as follows:

1956—Connecticut
1957—Oklahoma
1958—North Carolina
1959—Illinois
1960—Utah
1961—Wisconsin
1962—Georgia

Colorado has a new Dairy Industry Dept. with E. K. MCKELLER serving as head. The dairy work was formerly in the Dept. of Animal and Dairy Industry.

A. J. MORRIS became head of the Dairy Department at Utah July 1, succeeding G. B. CAINE, who has reached the retirement age.

Farrall Given Honorary Degree

A. W. FARRALL, head of the Dept. of Agricultural Engineering at Michigan State College, received an honorary Doctor of Engineering degree at the Univ. of Nebraska commencement in Lincoln June 13. Nationally recognized as an educator and research director in commercial engineering, Professor Farrall returned to his native state to receive the honor. He graduated from the Univ. of Nebraska with a B.S. degree in agricultural engineering in 1921 and an M.S. degree the following year. He did additional graduate work at the Univ. of California.

From 1932 until the time he joined the staff at Michigan State as professor and head of the Department, he did commercial research work in dairy manufacturing. He developed numerous pieces of dairy plant equipment and has written a college textbook, *Dairy Engineering*.

National Dairy Council Announces Date of 1956 Annual Meeting

Jan. 30-31 and Feb. 1 are dates selected for the 1956 annual meeting and winter conference of National Dairy Council, it is announced. Location will be the Roosevelt Hotel in New York City. Host to the meeting will be the Greater New York Producers' Dairy Council Committee, under whose sponsorship National Dairy Council conducts the Greater New York Dairy Council Program.

Attendance is expected to draw visitors from coast to coast. An especially large turn-out of producers and other industry people should come from the northeastern states, since 30 local Dairy Council offices are located in seven surrounding states. New York City, the country's largest milk market, is supplied by a milk shed of 37,000 farmers.

Members of the hospitality committee are: *president,* ELMER MCCANN, Chateaugay, N. Y.; *vice-president,* CLARENCE LITTLE, Sussex, N. J.; *secretary-treasurer,* J. B. HOLLOWAY, Amsterdam, N. Y.; O. F. ROSS, Lowville, N. Y.; A. M. CHENEY, Dairymen's League Coop., New York City; R. P. KINNEY, Syracuse, N. Y.; J. A. ROEBUCK, Walden, N. Y.; and PRESTON ERWAY, Genessee, Pa.

A National Dairy Council film recently won a Golden Reel Certificate of Acceptance at the Golden Reel Film Festival held in New York. The award is made by the Film Council of America. The color film, "It's All in Knowing How," is the most recent film production of N.D.C. and is aimed at correcting food habits in teenage youngsters.

It has been shown to an estimated 20 million persons since it was completed less than a year ago. It has had extensive use through the 71 local Dairy Council units throughout the United States. It has also been shown widely over television and in private showings through Association Films, Inc., an educational film booking agency with offices in Ridgefield, N. J., San Francisco, Chicago, and Dallas.

The teenager nutrition message is skillfully woven into a plot to show that correct eating habits, in contributing to health and appearance, help to ease social and family relationships in youngsters. The film points up the contribution made by dairy foods in the diet.

Milk Prices in West Virginia to Be Studied

The 1955 West Virginia Legislature created a commission for the purpose of studying the current problem of milk prices. The commission, appointed by the Governor, includes J. B. MCLAUGHLIN, Commissioner of Agriculture, *chairman;* S. J. WEESE, Dairy Dept., W. Va. Univ., *secretary;* and representatives of the Dairymen's Assoc., Dairy Cooperative Sales Assoc., and Farm Bureau, and the chairmen of the House and Senate committees on agriculture. Dairy processors and distributors also have been invited to have a representative sit with the committee. The commission is to make a complete and thorough study of all phases of the current problems of milk prices to producers; handling, processing, and transportation costs; retail prices; milk sanitation regulations and procedures; importation of milk products from other states; and any other relevant problems.

Cooper New Extension Head

J. E. COOPER of Blue Springs, Mo., has been named director of extension with the Holstein-Friesian Assoc. of America, filling the vacancy created by the retirement of G. M. HOUSEHOLDER. His headquarters will be Brattleboro, Vt.

Mr. Cooper brings to the Association a strong background of experience in the administration of agricultural extension programs and supervision of organized activities. He is at present assistant manager of the Adams Dairy Co. of Kansas City, Mo., a large distributor supplying fluid milk to chain stores, where he is responsible for procurement and processing of milk from more than 300 producers and directs all producer relationships for the Adams firm. He is a former fieldman with the same organization and is known for his work in developing pasture and silage programs on producer-owned farms. Mr. Cooper is also manager of the Adams Dairy Farm and part owner of the Holstein herd of over 100 head. A graduate of the Univ. of Missouri, he is a former assistant county agent and dairy extension agent in Jackson Co., Mo.

D. A. Pettee to Be Milk Judge in Contest

R. WHITAKER, National Dairy Research Labs., who has served as milk judge in the Students' Intern. Judging contest since 1947, has resigned because of the press of other duties. He is being replaced by D. A. PETTEE of the Creamery Mfg. Co., according to C. J. BABCOCK, Contest Superintendent.

The contest, held annually under the sponsorship of A.D.S.A. and DISA, has four judges —one for each product to be judged. Judges for the other products are: Cheese—H. L. WILSON, Kraft Foods Co.; Butter—N. E. FABRICIUS, Ladysmith (Wis.) Milk Coop.; Ice Cream—J. HOFFMAN ERB, The Borden Co., Columbus, Ohio.

Top awards in the contest are three cash fellowships, worth $1,380, $1,280, and $1,180, awarded by DISA. Other awards include silver cups and gold, silver, and bronze medals awarded by Intern. Assoc. of Ice Cream Mfrs., Milk Industry Foundation, American Butter Institute, National Cheese Institute, and DISA.

The next Collegiate Students' Intern. Contest will be held in late October in St. Louis in conjunction with the regular annual conventions of Milk Industry Foundation and Intern. Assoc. of Ice Cream Mfrs. and stand-by convention activities of DISA.

Plans Announced for Chicago Dairy Show

Premium lists, totaling $40,000 in cash prizes, are now available for the 3rd annual Intern. Dairy Show and Championship Rodeo to be held in Chicago's Intern. Amphitheatre October 8-15. Highlighting the exhibition will be nearly 2,000 head of the nation's top purebred cattle,

which will compete for top honors among the six dairy breeds. At last year's show prize animals from 28 states, Canada, and Cuba competed for the coveted awards.

Several new features have been added to this year's events. Quarter horses, the West's most popular breed of horse for ranch and farm work, will compete in halter classes, and cutting horse and reining contests also will be featured. These events are sponsored by the American Quarter Horse Assoc. and the National Cutting Horse Assoc.

Another new event is a Mid-October Feeder Cattle Show and Sale held in nearby pens of the Chicago Stock Yards. The feeders will be judged on October 13 and will be sold at auction October 14.

A $25,000 Golden Dairy Recipe Contest, already in progress, will be climaxed at the Dairy Show. The grand prize to the winner submitting the best dairy product recipe is a week's trip to Paris, sponsored by the American Tourist Bureau. Other prizes include refrigerators, washers, stoves, and many household appliances. According to RUTH FISHER HOLBROOK, food consultant for the Intern. Dairy Show, who is in charge of the contest, a dairy food forum featuring the demonstration of prize dairy recipes will be held twice daily in the show's "Dream Kitchen." Valuable door prizes and gift boxes will be awarded at each of these programs.

For spectacular entertainment there will be 14 world championship rodeos during the 8-day run of the Dairy Show. GENE AUTRY and his famous horse, Champion, will appear at each performance.

Drake to Become President of Pennsalt

Executive vice-president WM. P. DRAKE succeeded G. B. BEITZEL as president of the Pennsylvania Salt Mfg. Co. on July 1. Mr. Beitzel will continue his association with the company as a member of its board and, in addition, will serve as chairman of the board of the Pennsalt Intern. Corp., a subsidiary.

At 42 years of age, Mr. Drake is the youngest president in Pennsalt's 105-year history, and with 21 years' service prior to assuming office will have longer company experience than all but one of his thirteen predecessors. He came to Pennsalt as a summer trainee while still an undergraduate at Bowdoin College, started as a maintenance worker at the company's Wyandotte, Mich., works and after 3 years of diversified production experience was assigned to supervisory duties in the chemical specialties department. In 1941, he became manager of this department and subsequently served as vice-president in charge of all company sales.

In 1952, Mr. Drake represented his industry in the Office of Price Stabilization as director of the Rubber, Chemicals, and Drugs Division. When Pennsalt decentralized its operations in 1954, he became general manager of the company's largest operating unit, the Industrial Chemicals Division.

Virginia Polytechnic Institute News Notes

E. J. FINNEGAN has been granted an educational leave of absence from his duties in the Dept. of Dairy Husbandry for 1 year. He will pursue graduate work leading to a doctorate at North Carolina State. His duties will be taken over by PAUL LARGE, a recent graduate of the department. Mr. Large received his M.S. degree in dairy technology at Ohio State and has had considerable experience in dairy processing plants in Virginia and Ohio.

A highly successful conference was held by the Departments of Dairy Husbandry and Agricultural Engineering May 24-25. Over 100 persons registered for the conference and heard speakers from V. P. I., the dairy industry of Virginia, and Cornell, Maryland, and Penn State Universities. Latest factual information was presented on the handling of bulk milk on farms. This method of handling milk is rapidly being expanded in Virginia.

G. C. GRAF appeared on a panel discussion entitled "An Integrated Approach to Pasture Evaluation" at the Southern Regional Pasture and Forage Crop Improvement Conference in Knoxville, Tenn., June 14-16. A paper on "What Are We Learning from the Study of Grazing Animal Behavior and from Palatability Studies?" was presented by W. A. HARDISON.

R. G. CONNELLY left the Dept. of Dairy Husbandry July 1 to become secretary-treasurer and manager of the Virginia State Dairymen's Assoc. and the Virginia Milk Producers Federation. He was extension dairyman at V. P. I. for 24 years. M. F. ELLMORE will take over the duties of Connelly on the extension staff. Dr. Ellmore has been on the dairy staff since 1952 and has handled the testing, feeding, and management project in dairy extension. He is a native of Virginia and received his B.S., M.S., and Ph.D. degrees from the Univ. of Maryland.

Louisiana Dairy Trends

Louisiana's dairy industry experienced a growth of 23% in number of milk cows between 1949 and 1955, according to B. B. JONES, agricultural manager of the Chamber of Commerce of the New Orleans area. During this period the national increase was 5%. The federal report showed Louisiana to be third in the percentage of increase. A survey shows that the number of producers in Louisiana is still declining, but production per producer is on the increase. The average price received by producers for Class I, 4% milk, in April, 1955, was $5.88 per hundredweight. The blend price for the same month was $5.19.

Diversey Appointments Announced

W. E. NOYES, vice-president in charge of sales of the Diversey Corp., has elevated three men to new posts created in the expansion of the company's sales program. J. A. MCMURTRY, formerly assistant general sales manager, has been named division manager to head a newly created East Central Division. C. R. REID has been promoted to sales manager, Food Industries, and H. M. PICKLES to assistant manager.

The Diversey Corp. is a manufacturer of specialized sanitation and maintenance chemicals for the food, beverage, and institutional fields.

Completed Theses

M.S. Degree:

W. F. AXELGARD—Use of corn syrup solids of different dextrose equivalents in the manufacture of frozen desserts. Utah Agr. College.

D. L. HAYS—The cost of raising herd replacements through twelve months of age under dry-lot conditions for Louisiana. Louisiana State Univ.

R. E. HOWE—A use of the hot brine treatment to make Cheddar cheese from "slow milk." Utah Agr. College.

D. A. HUTCHISON—Some aspects of reproduction in the West Virginia University herds. West Virginia Univ.

J. B. LINDAMOOD—A study of detergents and detergent-sanitizers used in the in-place cleaning and sanitizing of milk pipe lines on the farm. Virginia Polytechnic Inst.

B. T. PARHAM—A comparison of cottonseed meal, urea and ammoniated molasses as a source of nitrogen in the ration of dairy heifers. Louisiana State Univ.

GEORGE PATTERSON—Lactation curves of Holstein cows as influenced by age, gestation, and season of freshening. Utah Agr. College.

MERTON POORE—Long-time storage of bovine semen. Univ. of Maine.

C. R. WINKEL—Effects on urinary metabolites of rats fed various edible fats. Utah Agr. College.

J. J. VIZINAT—The influence of elevated temperature and humidities on lactating Holstein-Friesian and Sindhi-Holstein (F_1) cows. Louisiana State Univ.

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Bovine Leptospirosis in the United States

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Bovine leptospirosis is a contagious disease of health significance to the public and of financial importance to the stock owner. Recent interest in this disease has stimulated research, and many publications have appeared pertaining to it. A review of some important features of bovine leptospirosis is presented here.

Canine leptospirosis has been recognized in the United States since 1937 *(6)*, but bovine leptospirosis was not diagnosed in this country until 1944 *(5)*. Recognition of the disease in cattle was sporadic until York *(9)* in 1952 introduced a practicable serological test for diagnosis. His publication stimulated national interest in the disease among veterinary and allied workers.

Cause and Transmission

Leptospira pomona is the principal cause of bovine leptospirosis in the United States. *L. sejroe, L. grippotyphosa, L. canicola,* and *L. hebdomadis* have been identified occasionally in cattle by serology. The severity of symptoms varies among species of the infecting organism, degree of exposure, and resistance of the infected animal.

Transmission is dependent upon the presence of animals "shedding" leptospirae in urine and body secretions. Contaminated water and moisture can prolong leptospiral viability and promote spread by droplet inhalation or skin abrasion contact. Repeated conjunctival and parenteral inoculation of the organism will consistently reproduce the disease experimentally. Leptospiral ingestion is considered to be innocuous because of abomasal acidity; however, four yearling calves were infected at this laboratory by allowing them to drink "shedder" urine-contaminated water when other routes of contact were excluded.

Symptoms

Bovine leptospirosis is characterized by two phases. After a 1- to 2-week incubation period, a leptospiremia develops, and symptoms at this stage are related to injury to the blood vascular system. If an animal survives the leptospiremia, then leptospirae localize in kidney, liver, and udder and uterine tissues, and symptoms are then attributable to organ injury.

The leptospiremic phase is usually severe in calves and yearlings, whereas it may be mild and unrecognized in adult cattle. Temperatures of affected animals vary from 104° to 107° F. for 1 to 3 days, and a hemolytic anemia develops. As hemolysis progresses, icterus and hemoglobinuria appear, and some animals become dyspneic. Anorexia, depression, and diarrhea are observed during this stage.

Adult cattle often show no illness until tissue localization of leptospirae has occurred 5 to 10 days after initial symptoms. Symptoms at this time may be either mild or severe, depending on the extent of the organ injury. Mastitis, manifested by a flaccid udder containing a thick yellow or blood-tinged milk; abortions, late in gestation, accompanied by frequent placental retention; and uremia are attributed to leptospiral activity in the involved organs.

Renal localization of leptospirae in affected animals produces a leptospiruria, and viable leptospirae are voided in the urine for a variable period from a few days up to several months. This "shedder" condition is believed to be primarily responsible for transmitting the disease to susceptible cattle.

Recovered cattle develop a long-lasting immunity to the infecting leptospiral serotype, but they are susceptible to other species of the organism.

Diagnosis

Leptospirosis is diagnosed by symptoms, by gross and microscopic pathology, by organism isolation from tissues or urine, and by serology. Serological tests (complement fixation, agglutination-lysis, agglutination, etc.) are popular for routine diagnostic purposes. Their accuracy is enhanced by performing two herd tests with a 2- to 4-week interval between tests to demonstrate a rising blood titre, which is indicative of recent infection. Blood titres persist for months to several years after an animal recovers from leptospirosis.

"Shedders" are identified by demonstrating a leptospiruria. This is accomplished either by

inoculating serologically negative laboratory animals with suspected urine and demonstrating their infection or by examining suspected urine for leptospirae by darkfield microscopy. Microscopy is rapid and efficient if numerous organisms are present, but small numbers may be overlooked.

Treatment and Control

Many antibiotics have been employed for treatment, but results have varied. No controlled studies have been made to evaluate treatment methods for acute bovine leptospirosis. Ringen *et al. (7)* reported that streptomycin (3- to 5-day regimen) is effective for eliminating "shedders."

Control is based on prevention of herd exposure. New acquisitions should be isolated and tested prior to herd entry. Since leptospirae are associated with moisture, adequate clean drinking water and proper table water drainage are essential to control transmission.

Several methods have been employed with varying success to control leptospirosis in an infected herd. Test and temporary segregation of reactors and clinically sick cattle until they are noninfectious is of limited value because some negative animals may be in the incubative stage and may give a false negative test. Vaccination for *L. pomona* is effective during early herd exposure, but vaccinal immunity requires 2 weeks to develop and may last for only 3 to 6 months. Where practicable, "shedders" may be identified by darkfield microscopy and treated with streptomycin. Also, streptomycin administered to new acquisitions during the isolation period may be of value in preventing herd exposure.

Leptospiral strains causing bovine infection have been identified serologically in horses *(8)*, sheep *(2)*, pigs *(4)*, and chickens *(3)* on farms during an enzootic. Also, some of these strains have been identified in various rodents and wild herbivora *(1)*. Control measures for cattle should include control of these infection reservoirs.

Leptospirae causing bovine leptospirosis are pathogenic for man. Precautions to prevent human exposure and infection should be observed.

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Efficiency in the Operation of Dairy Plants

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There has been an increasing trend toward more governmental control in business. Public endorsement of this policy was particularly emphatic in the 1932 and subsequent national elections. This philosophy apparently has not changed greatly since that time. Many people in the dairy industry today are convinced that present marketing difficulties had their origin in excessive governmental controls that were exercised during the World War II period. Since some dairy products may be found in almost everyone's diet, the affairs of the dairy industry have always been extremely newsworthy. Since dairy products make up 15 to 30% of the food budget, any change in the price of milk or other dairy products is important to the budget-conscious housewife. Even a moderate number of complaints by housewives can bring about a congressional investigation.

Newspaper readers have been greeted recently by headlines such as this: "Congressional committees to examine increased margins in dairy profits." The U. S. Senate Agricultural Committee has urged that "the majority of processors of milk and dairy products re-examine their operating costs and profit and loss statements and take action to reduce marginal spreads in prices as well as prevent a further spread of this margin." The implication is quite clear. The committee apparently assumes that profit margins are too great.

The approach which the committee has taken

is important because if margins are to be narrowed, this must be accomplished through operating economies. The committee implies that it can be done by reducing profit margins. This involves a judgment on business practices. Stockholders in a corporation are entitled to a fair return on their investment. The dairy industry, as a whole, has been returning 50% of its net profits, if any, after taxes in the form of dividends to its stockholders. This means that presently the returns are in the neighborhood of 1% per dollar sales. On the basis of the net worth of an organization, this amounts to approximately 5.5%. The two general methods have been used by industry for reporting profits. The National City Bank of New York has for many years annually published a table which, among other things, shows these two profits for many groups of industries. Presumably, the more than 3,000 companies represented are those who are required by the Security and Exchange Commission to file annual financial statements. This leaves out thousands of concerns whose stock is not traded on any of the big exchanges. The approximately 15 dairy companies included in the National City Bank report are the largest and presumably among the best managed in the nation. Space will not permit a profit comparison of industry by industry, as has been done in the Bank's bulletin, but the figures for the dairy companies can be compared with those for all industries shown. This has been done in Table 1.

TABLE 1

Average profit, after taxes, compared on a net-worth and on a gross-sales basis for 15 leading dairy corporations and for more than 3,000 business corporations (including the dairy companies).

Year	Dairy corporations profits		All corporations profits	
	On sales	On net worth	On sales	On net worth
	(%)	(%)	(%)	(%)
1940	3.2	9.2	7.5	7.8
1941	2.9	10.2	6.5	8.9
1942	2.3	10.1	4.3	8.5
1943	2.2	10.3	3.6	8.6
1944	2.1	10.7	3.9	8.2
1945	2.1	18.9	3.9	7.7
1946	3.2	18.8	6.0	9.5
1947	2.5	15.2	6.8	12.3
1948	2.4	13.0	7.3	13.6
1949	3.2	15.2	6.6	11.0
1950	3.0	13.9	7.7	13.4
1951	2.2	10.8	6.2	11.4
1952	2.1	10.4	5.6	10.4
1953	2.2	11.0	5.6	10.5

It can be seen that profits on sales in the dairy industry are relatively low but that profits on net worth are high when compared with other industries. The quick turnover of products in the dairy industry is responsible for these differences. However, because of this quick turnover, risks are greater and the slightly higher profits on net worth appear justifiable.

It is unlikely that a congressional investigating committee will spend much time on small organizations, such as a cheese factory at Cross Roads Center, U.S.A., or on a one-route milk plant at Podunk. We might well ask ourselves if the committee will recommend that the dairy industry follow in the footsteps of the automobile industry. May we ask if it is in the best interests of the nation to process all dairy products through a few large corporations?

The price of a quart of home delivered milk has been within the range of 12 cents in 1925 to roughly 24 cents at present. In the same period the price of certain well-known popular automobiles has increased from $600 to $2,400. The improvement in the quality of the $600 automobiles has not been greater in this 30-year period than the improvement in the quality of the quart of milk which is presently placed on our doorsteps.

Notable improvements in the economy of milk plant operations in recent years have helped to keep costs of fluid milk low. Most important of these improvements is every-other-day or three-day-per-week delivery of milk. Another is the fairly rapid shift from home delivery to store sales. What are some other practices which management can undertake to improve efficiency?

The Approach

First it will be necessary to overcome prejudices associated with efficiency programs. Mention "efficiency" or "efficiency engineering," and there are a variety of reactions. Neither term has been popular with labor or management. The erroneous belief has persisted in both groups that efficiency involves either lay-offs or speed-up of workers. This is true in isolated instances only. Some years ago efforts were begun to remove the opposition to the word efficiency by substituting "technocracy." However, there was no general acceptance of the term. As greater specialization came into the picture, mention was often made of "time and motion studies," "work simplification," and "automation." To this group might be added "conservation of procurement, production, and distribution." The first two terms relate directly to personnel management. The third involves the application of automatic control techniques to machine and factory operation. The fourth is self-explanatory. Actually, there should be no valid objection to the term "efficiency" when it is used in connection with cost reduction by the use of more effective application of available facilities. Efficiency engineering has been defined as "a study of business management with a view toward more effective operation with a minimum amount of effort,

and the elimination of waste, all with the aim to reduce costs."

Numerous more or less specific proposals have been made for increasing the efficiency of plant operations. In a paper such as this, only a general outline is possible. Under some conditions, use of an efficiency engineer or an industrial firm specializing in this field may be the quickest and best solution for plants suffering from lack of efficiency in operations. Many such organizations prefer to specialize. Where they work with steam, water, or power, their fees are often based on a percentage of savings which they are able to show over some previously selected period. When a survey of this kind is contemplated, management should ascertain if the study can be conducted by its own personnel. It should assure itself that the savings indicated can be maintained after completion of the survey. No matter what the approach may be to a cost reduction program, personnel will continue to be the key factor. Unfortunately, no one has developed a formula or an equation to predict human reactions to proposed ideas and plans.

There are two general procedures for reducing costs per unit by increasing efficiency. One is a direct approach to labor output. The other is indirect. The first has to do with increased output per man-hour, whereas the second deals with saving of wastes, better quality control, better composition control, more uniform weights, improved manufacturing practices to increase yields, improved sales practices, etc. Those who aim to introduce new or improved procedures in order to reduce production costs need not necessarily be trained efficiency engineers. Often all that is needed is the application of sound common sense. The ability to cooperate with fellow workers is highly important. Before initiating any cost reduction program all who will participate should be "briefed" on the need for the survey and the objectives which are sought.

Reducing Costs by Increasing Output per Man-Hour

The proper use of man-hours in the dairy plant has always been recognized as important. In a recent address presented at the Intern. Dairy Show in Chicago, Secretary of Agriculture, EZRA TAFT BENSON, pointed out that wages make up a high percentage of milk distribution costs but that the problem of high labor costs "lies not so much in high wages, as such, but rather in the failure to increase output per man-hour." The Secretary did not cite specific data and apparently assumed that his statement was not subject to challenge. U. S. census figures, as far as they go, appear to bear him out, with respect to workers in the dairy manufacturing field, as shown in Table 2.

The latest production figures obtainable from the U. S. census are for the year 1947. The figures in Table 2, however, indicate that there has been but a minor improvement from 1909 to 1947. Data, of course, do not reflect the extra services which have been demanded by consumers and regulatory bodies in recent years. Among these extra services are quality field work, improved and more costly manufacturing practices, such as factory separation of milk in creameries, pasteurization of milk in cheese factories, more packaging services, and better sanitation. Since the data in Table 2 are on the basis of yearly output, the present shorter work week or work year should not be overlooked. In general, the work year has decreased from a high of 3,600 to approximately 2,000 hours at present. Data obtained from various sources for fluid milk plants appear to bear out the contention of the Secretary of Agriculture. In Table 3 the range in output per man-hour of plant labor for milk plants for a series of years is indicated.

The results shown in Table 3 are subject to some criticism. The plants are not all in the same size group. This is true in particular for the two plants shown for the year 1946. Furthermore, these two plants had been selected for a study because of their high efficiency. They should, therefore, be regarded more or less as an ideal objective rather than as an average. The data in general present some evidence that output per man-hour has increased since 1922. It should be pointed out that 10 of the plants in the 1952 group were operating under a well organized accounting system.

TABLE 2

Average yearly output per wage earner and supervisory person in three branches of the dairy processing industry

Year	Cheese		Butter		Evaporated and condensed milk	
	Product	Index[a]	Product	Index	Product	Index
	(lb.)		*(lb.)*		*(lb.)*	
1909	103,159	90	40,243	66	99,717	38
1919	103,107	90	34,932	58	122,843	47
1929	114,313	100	60,679	100	259,667	100
1939	102,106	89	72,092	119	232,009	89
1947	114,004	100	64,650	107	230,472	89

[a] The index for output is placed at 100 for the year 1929.

TABLE 3

Output per man-hour of plant processing labor

Year	No. of plants	Av. amt. processed daily	Av. amt. processed per man-hour[a]	Range, where shown
		(lb.)	*(lb.)*	
1922	4	148,350	216	
1932	65	97,371	243	229-286
1943	10	67,211	229	94-420
1944	11	15,480	198	
1946	2	107,543	593	
1948	58	16,416	201	155-239
1949	10	18,099	176	
1952	14	25,130	332	172-570

[a] This does not include maintenance, laboratory, or power plant labor but does include checkers, refrigerator help, load-out men, and plant janitors.

In an address before the annual convention of the Milk Industry Foundation in Boston, Oct. 27, 1953, R. E. Olson, agricultural economist, Division of Marketing and Transportation Research, USDA, stated that the output per man-hour of plant labor amounted to 194 lb. in plants handling 11,000 lb. of milk per day and that it increased at the rate of about 21 lb. per man-hour for each increase of 10,990 lb. per day. It may be assumed that these increases apply only within certain limitations.

In reported studies of output per man-hour of plant labor in fluid milk plants, individual plants show variations ranging from 155 lb. to 600 lb. Without regard to time, the reported average output of groups of fluid milk plants arranged according to size is shown in Table 4.

TABLE 4

Milk processed per man-hour of plant labor in fluid milk plants

No. of plants	Range in size	Av. amt. processed daily	Av. amt. processed per man-hour
	(lb. per day)	*(lb.)*	*(lb.)*
16	Under 5,000	2,924	164
16	5,000- 10,000	7,309	213
38	10,000- 20,000	17,217	199
28	20,000- 40,000	27,560	246
44	40,000- 80,000	61,961	250
25	80,000-160,000	124,020	280
8	Over 160,000	267,176	284
Av.		54,593	233

Suggested Procedures for Increasing Output per Man-Hour

Those whose duty it is to decrease operating costs look upon successful practices in other plants in the same or related industries as a logical starting point. This is good reasoning, provided they realize that it is not usually possible to adopt an existing plan without making some changes. Efficiency in operations is often achieved by a rearrangement of old ideas into new patterns or combinations. Every organization has a right to expect its executives to think, but it must provide them with the time to think.

Every new machine, every arrangement, every idea or plan is the result of human effort or human thought. At the same time, human beings—employees—must live and work with the machinery, the layout, and the people who are responsible for the new ideas. For this reason it is usually wise to limit changes at one time to only part of the operations or to a particular department. Experimentation with so-called "systems" is dangerous when undertaken on a large scale.

The concept of incentives has changed materially since World War II. Daily wants have increased. A citation or an emblem alone no longer achieves the desired response. Prestige or material awards are more productive of results. Improvements must be measurable if equitable awards are to be made. On the other hand, the contributor of an idea must understand that the opportunity to develop his ideas was possible primarily as a result of his employment. In this respect he does not differ from the self-employed individual. Some organizations, as well as some educational institutions, include as a condition of employment a provision that any patentable or copyrightable equipment or ideas belong to the employer or at least must first be offered to him. However, such contracts often include clauses providing for suitable personal remuneration. Such remuneration usually does not exceed 10 or 15% of the net profits realized from the patents or copyrights. Awards may also be offered when no patent or copyright is involved. It is then usually a lump sum determined by a board or committee representing both the employer and the employees.

In order to increase personnel efficiency, an employer should establish a policy for promotions. Unless the organization is very large, it is not always possible to find the right man without going to the outside. However, it is poor practice to overlook likely candidates within the organization, no matter how small the company. Every attempt should be made to encourage employees to engage in self-improvement programs. Attendance at conferences, conventions, or schools should be encouraged and should not be looked upon as a vacation. If expenses are paid in part or in entirety, an oral or written report should be requested. If such reports are made to the staff of a specific department or to key men in an organization, the contents can then be evaluated and aspects developed which otherwise might be overlooked.

An organization should not overlook the recruitment of young employees with future possibilities for advancement. If the company is

large, a careful balance must be maintained between technical and nontechnical help. High school graduates should be encouraged to give more consideration to careers in the dairy industry. Since a dairy company frequently controls its products from the producer to the consumer, there is opportunity for almost every type of talent. It is the duty of management to match the man with the job if maximum output is to be expected.

Material awards in the dairy industry are not inconsiderable and compare favorably with those in other agricultural fields. In a recent survey by the University of Illinois[1] of jobs and salaries of 2,547 of its Agricultural College graduates engaged in 58 job classifications, those in the dairy industry ranked 9th in salary and exceeded the average of all by $2,620 per year, and they exceeded the average of the 650 who were engaged in business or industry by $941 per year.

Reduced output per man-hour can be the result of improper care or maintenance of equipment. Breakdown of a one-man machine, such as a can washer, a conveyor, a bottle washer, or a bottle filler, idles not merely one person but in many cases an entire crew. It has been estimated that ten men being idle for 2 hours reduces output per man-hour 1%. Improper scheduling of operations, such as straggling loads of incoming milk, or improper sizing of equipment is responsible for reduced output per man-hour.

Effective use of machinery as well as its proper arrangement in a plant will help to increase output per man-hour. In one fluid milk plant doing a yearly business of $2,500,000 a saving of $20,000 per year in wages for processing was made possible by a rearrangement of equipment at a cost of less than $4,000.

Newer methods of cleaning, such as the use of pressure systems or cleaned-in-place practices, have been shown to reduce cleaning time of equipment by 75 to 80%. Additional savings are often possible on cleaning materials used in the process. "Automation" has been suggested as a means of increasing output per man-hour. Unless men are released by such automatic equipment to take care of other work, there will be no savings, and "automation" may even increase over-all costs. It should help to produce a more uniform product.

If the output per man-hour is increased in a plant, a surplus of man power is likely to result. If these men are retained without increasing total output, no savings can occur. In some instances this problem can be handled as a result of normal job mortality. Social Security and established pension plans make possible earlier retirement without hardship. To some extent the dairy industry is seasonal, and a portion of the labor requirement is of a transient nature.

[1] Journal of Dairy Science, 38: 105, January, 1955.

When it is not feasible to discharge surplus labor resulting from increased efficiency, it is imperative that employees be shifted to another department and not retained in the department where the saving has been made. Most plants can find work to be done which for various reasons has been neglected or overlooked.

Increasing Efficiency by Other Means

From the definition of efficiency as previously proposed it is clear that production costs per unit of profit may be reduced by other means as well as by increased output per man-hour. Employees readily recognize that reduction of losses, wastes, and supplies will not jeopardize jobs. As a matter of fact, such savings promote job security because costs are reduced and the company is placed on a more sound financial footing. Standards for losses have been established. More research is necessary in order that such standards may be accepted as accurate and representative. Presently they often represent opinionated guesses rather than proven factual information.

Wastes and losses are not confined to the milk solids. They may include unnecessary use of lights, particularly in cold rooms. Not only the extra electricity, which is relatively cheap, but extra refrigeration, which is expensive, increases costs. In addition, dairy products that are packaged in clear glass containers may be damaged from the standpoint of quality when lights are turned on for excessively long periods of time.

Too many plant operators make no effective attempt to balance product output against product input. Even well managed large organizations are not satisfied with either their methods for checking plant losses or the results which they are obtaining. Sometimes procedures are at fault. Recently a large plant reported a loss of fat in its bulk pick-up operations, but it had a gain on its product output. It was possible to trace this apparent inconsistency to the use of the Babcock test for certain checking operations, whereas the Mojonnier test was used for others. Even within the orbit of the Babcock test for different products the comparison between it and the ether extract method will vary.

A lack of attention to attainable yields or an improper composition of a product can result in a costly loss, especially in small cheese factories or creameries. This has been partially compensated for in the cheese industry by paying for natural cheese upon a moisture basis. A thorough knowledge of possible yields may serve to alert management to unbusinesslike practices, carelessness, lack of knowledge, or even larceny on the part of its employees.

Overweight of packaged products may be due to carelessness, to lack of technical knowledge, or to antiquated systems insisted upon by law enforcement officials. For example, it is well known that glass containers for milk are cali-

brated at 68° F. Yet they are filled with milk at 37°-40° F. The difference in weight of a given volume of 3.5% milk at 68° and at 38° F. is enough to result in a weight loss of 0.41% in bottle filling operations. The known difference in the weight of milk at different temperatures should be considered when calibrating bulk tanks and when filling paper containers. Furthermore, milk is purchased on a weight basis but is sold on a volume basis.

Improving the quality of products should be regarded as an efficient practice because it lessens the number of returns and the number of complaints that must be handled. Improved quality can be responsible also for increased number of sales and, therefore, more efficient operation.

A Basis for Measuring Efficiency Should Be Established

Astonishing as it may seem, many operators of small dairy plants do not know from one year to the next whether they are making a profit or earning a living wage. The correction of this situation should be the first step in an efficiency program. The second step should include some system for ascertaining operating costs per unit of product. Without a satisfactory accounting system, such items as depreciation of capital assets may be overlooked. The company may lack adequate working capital, or the ratio of assets to liabilities may be such that it cannot obtain bank credit. As a result, it is unable to take advantage of quantity discounts when purchasing supplies, and it may be compelled to operate with obsolete equipment. An improper inventory system may result in overstocking certain items, thus tying up working capital.

Before management can ascertain whether or not it is efficient, some guide or standard must be developed by which improvements can be measured. Such a guide is somewhat analogous to a budget system with respect to business operations. Some dairy companies (mostly relatively small cooperatives) publish detailed yearly operating statements. Such statements may serve as a guide for comparisons provided they are standardized in form. If rather wide cost differences are apparent on specific items, an attempt should be made promptly to ascertain the reasons for the differences. Satisfactory comparisons are possible where operating practices and policies are similar.

A considerable number of agricultural schools, some schools of business, federal agencies, and private organizations have investigated and published operating cost data in various forms for dairy plant operations. Time and motion study data are becoming increasingly available. These sources can be freely drawn upon.

In recent years, several research groups have taken an altogether different approach to studying costs of operation. These approaches involve the development of theoretical operating data in order to establish standards of operation.[2]

It was pointed out that operating statements from smaller plants are available for comparison. Large dairy organizations also publish more or less comprehensive annual reports. These can serve as important guides for measuring business efficiency. Ratios which have been worked out from such statements for this purpose may indicate reasons why the cost of a unit of product varies from that of a successful competitor. A series of such ratios for a group of large dairy corporations operating on a national basis is shown in Table 5.

TABLE 5

Significant average operating ratios of 12 large dairy companies for the years 1947 to 1951, inclusive

Items of comparison	*Dollars*
Profits per dollar of sales	0.0268
Profits per dollar of net worth	0.1313
Profits per dollar of fixed assets	0.1811
Profits per dollar of working capital	0.2104
Dividends per dollar of profit	0.4929
Dividends per dollar of book value	0.0706
Net worth per dollar of total liabilities	1.4965
Net worth per dollar of working capital	1.5994
Net worth per dollar of capital stock	2.4133
Fixed assets per dollar of sales	0.1473
Fixed assets per dollar of total assets	0.4331
Fixed assets per dollar of net worth	0.7254
Fixed assets per dollar of profit	5.5218
Working capital per dollar of sales	0.1273
Working capital per dollar of net worth	0.6252
Working capital per dollar of cash	2.3975
Current assets per dollar of current liabilities	3.2100
Accounts payable per dollar of sales	0.0407
Accounts receivable per dollar of sales	0.0451
Depreciation per dollar of fixed assets	0.1022
Depreciation per dollar of sales	0.0144

No plant can establish efficient operations unless its efforts are persistent and continuous. A careful survey must be made in order to determine the kind and the extent of reports desired. Only after such study should an attempt be made to develop the proper reporting forms. Modification should be made promptly if pertinent data have been omitted or if irrelevant data have been included. Useless reports should be abolished. Someone should be held responsible for summarizing data. Management must show an interest in the reports; otherwise they become meaningless and serve no useful purpose. Periodic comparisons should be made of all reports submitted; the shorter the period for the comparison, the more effective will be the results. The dairy industry is a fast moving business and yearly comparisons are inadequate. One of the better dairy corporations, profit-wise, carries an over-all inventory only slightly in excess of 4% of its yearly

[2] Northeast Regional publ. No. 16 or Virginia Polytechnic Institute, Agr. Expt. Sta. Bull. 463, Blacksburg.

gross sales volume. Such narrow operating tolerances would be impossible without adequate records and their daily observation.

A Summarized Program

When an attempt is made to increase the output per man-hour, extreme care in operation is necessary in order to obtain the proper cooperation from employees. The procedure would be somewhat as follows:

1. Obtain basic data to demonstrate the need for the study. The results to be obtained must be obvious; otherwise employees will become skeptical.
2. Carefully study all presently required reports to ascertain their need and add only such as will contribute useful and needed information. Eliminate all useless reports.
3. Break the plant up into work centers, and insofar as possible handle each center as a unit when making efficiency studies. Make the study in one center at a time.
4. Start a time-study plan by establishing "fixed" time for a job, e.g., assembling a machine, making change-overs in filling equipment, etc.
5. Provide for proper operating controls and regular reports to supervisors in order to guide them in their recommendations.
6. Have a prepared list of "special" work to which employees may be assigned if standard operations are completed before the close of the work day.
7. Develop objectives with the supervisors and employees so they will understand the purpose for the study.
8. Emphasize improved methods and proper work loads so that employees understand that they are not being asked to do more than is reasonable.
9. See that good working conditions are provided, such as proper lighting; comfortable positions, clothing, and temperature; non-skid floors, and pleasing color schemes.
10. Have an organized plan for doing a given job in the easiest, fastest, and safest possible method.

In order to establish profitable operating and business procedures, it is necessary to ascertain whether wastes or losses are excessive. This must be done in all categories, including the product and the materials used. Accurate laboratory control is necessary in order to check on composition and quality. Output should be checked against input. Employees should be encouraged to submit ideas by providing for proper compensation. Plans for such compensation should be very carefully developed before they are initiated. If a bonus system is introduced, it should be practical and understandable and of such a nature that it will not encourage dishonesty.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

432. The pathogenicity of certain strains of Escherichia coli for young mice and calves. T. MOLL and C. K. INGALABE, Wash. Agr. Expt. Sta., Pullman. Am. J. Vet. Research, **16**, 59: 337. 1955.

Nine strains of *E. coli* isolated from diarrhea and enteritis cases were fed to suckling mice and calves. The calves had received colostrum for 24 to 48 hr. before put on experiment. None of the strains produced clinical effect on the calves. Two of the strains caused death of all of the treated mice. Three of 7 calves had serum agglutinins against *E. coli* before treated. All had agglutinins after treatment. The role of immunological protection of the calves was not determined conclusively.

E. W. Swanson

433. Studies on bovine mastitis. II. Methods of diagnosis. T. NARAYANAN and K. K. IYA. Indian J. Dairy Sci., **1**, 147. 1954.

The efficiency of various indirect tests (digital palpation, strip-cup, indicator paper, chloride content, rennet coagulation, reazurin test, leucocyte count, Hotis test) was compared with the diagnosis based on bacteriological evidence. The microscopic examination of Hotis test incubated samples and the Hotis test itself were considered the two most reliable indirect tests followed by the leucocyte count; the strip-cup test was considered the poorest. A chloride content in excess of 0.1% and a leucocyte count of more than 500,000 cells per ml. was considered as indicative of infection.

W. C. van der Zant

BOOK REVIEWS

434. Radioisotopes in Biology and Agriculture—Principles and Practice, by C. L. COMAR, McGraw-Hill Book Company, Inc., 481 pp. $9.00. 1955.

The subject headings and page space allotted to each subject follow: Principles of tracer methodology (59), Basic difficulties in tracer methodology (25), Health physics and radiation protection (32), Facilities and handling of radioisotopes with animals and plants (45), General procedures for radioassay, Properties and procedures for individual radioisotopes (44), Autoradiography (37), Paper chromatograph (28), Ion exchange (27), Radioactive analysis (15), Glossary of selected terms in nuclear science (18), Availability of U. S. Atomic Energy Commission reports (2), Summary of available radioisotope preparations (8), name index (13), and subject index (7).

An objective of the author taken from the preface reads: "A primary purpose of this volume is to bring home to the student and investigator an appreciation and understanding of how radioisotopes can fit into his program and then to show how the experimental work can be undertaken." The decided emphasis is to a discussion and evaluation of specific procedures. This objective of the author has been achieved further through a consideration of many of the practical details necessary for conducting experiments employing isotopes. In addition, such procedures as wet ashing are rather thoroughly discussed under general procedures. It may be noted that the text includes chapters concerned with paper chromatography and ion exchange. These chapters include references to many of the more useful procedures employing these techniques. The information is presented in a systematic and a well documented manner.

The reader will find much material of general interest that is easily read, for example in the chapter "Principles of tracer methodology" under a section heading concerned with miscellaneous applications one can find reviewed the classic definitive experiments of Potter and Heidelberger on citric acid metabolism and a discussion of the problems encountered in the radiocarbon method of age determination.

Many theoretical considerations are not found in this treatise, however, ample reference is made to both general sources and specific papers (143 references to chapter 1 for example). The bulk of the examples which the author chose to consider are concerned with animals. The plant biochemist will find the text useful as reference plant materials have not been ignored.

R. G. Hansen

435. Practical Cheesemaking—8th Ed. G. H. WILSTER, OSC Cooperative Association. $7.00. 1955.

This book is a revised and enlarged edition of the author's previous text. It is printed by an off-set process and bound in loose-leaf style with glossy paper covers.

In the preface, the author defines his objective: "to assemble essential and reliable information dealing with the practical manufacture of cheese." It, therefore, is a book designed for the apprentice cheesemaker and not for the college classroom.

The book is divided into seven parts with the pages numbered consecutively in each part. Part I contains material of a general nature related to production of milk, grading and sanitary standards for milk, and sanitary practices within the cheese factory. Part II lists tests for quality and composition of milk, preparation and use of starter, rennet, cheese color, and standardization of milk for cheese. Part III gives a compilation of material related to filtering and clarifying milk, pasteurization of milk, HTST method of pasteurization, and vacuum pasteurization of milk. Part IV contains the material which pertains to Cheddar cheese manufacture and related topics—such as, factors affecting yield; testing hard cheese for fat, moisture, and salt; washing and caring for milk cans and cheese making equipment; mold control; loss of weight during curing, packaging cheese in transparent materials, etc. Part V has brief outline directions for making about 30 different named cheeses. Part VI lists various uses for whey and brief outlines for manufactured products from whey. Part VII lists federal and state standards for composition of dairy products and federal definitions and standards for cheese.

The fact that the book is a compilation of material from many sources, prevents it from having the unity of style and sequence that one would expect to find in a text book. Nevertheless, there will be many workers in the cheese industry who will find this book useful.

S. L. Tuckey

BUTTER

436. Bepaling van het luchtgehalte van boter (Determination of air content of butter). G. Posthumus. Off. Orgaan van de F. N. Z., **46**, 904. 1954.

A method is described to determine the air content of butter based on the specific gravity of butter. A cylindrical piece of butter is weighed and then placed on the point of an aerometer under water (13° C.). The aerometer which without butter sank in the water to a set mark has to be reloaded with weights to bring it to the same position. A detailed description of the aerometer and test procedure is presented. W. C. van der Zant

437. Studies on the production of ghee (clarified butterfat) directly from cream. II. The qualitative factors in cream affecting its washability for reduction of serum solids. G. S. Mani, M. R. Srinivasan, and S. C. Ray. Indian J. Dairy Sci., **7**, 69. 1954.

Cream of 40% fat gave the highest yield of ghee. Neutralization of high acid cream followed by washing did not offer any advantage over simple washing of high acid cream.

W. C. van der Zant

438. Vitamin A in dairy products. II. Vitamin A in curd and ghee from fortified milk. K. M. Narayanan, N. B. Shroff, C. P. Anatakrishnan, and K. C. Sen. Indian J. Dairy Sci., **7**, 76. 1954.

Oil soluble and water-miscible forms of vitamin A used for fortification of milk were not affected by souring. During the preparation of ghee from milk fortified with water-miscible form became soluble in cream, creamery and desi ghee resp. By direct cooking of fortified cream practically all the water-miscible vitamin A was recovered in ghee.

W. C. van der Zant

439. Method of manufacturing butter. H. A. Toulmin, Jr. (assignor to The Commonwealth Engineering Co.). U. S. Patent Reissue 23,988. 13 claims. Apr. 26, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 4: 414. 1955.

A revision of Patent 2,505,654 (see J. Dairy Science Literature Abstrs. No. 554, Vol. 33, No. 8, A111). R. Whitaker

440. Quality and pricing of butter manufactured from farm-separated sour cream. E. Feder. Milk Prod. J., **46**, 6: 26. 1955.

The sporadic buying of higher grades of butter by the government under the price support program, the need for storing it over prolonged periods of time, and the decline in demand for butter, focus attention sharply on the problem of quality and storability of butter produced from farm-separated sour cream.

The author is concerned primarily with South Dakota conditions where 90% of the milk produced by farmers is marketed as farm-separated cream. The discussion covers methods of procurement and how they affect the quality of output of butter plants. Quality is discussed from the standpoint of Federal grades and chemical and physical tests.

The conclusions point to the need for farmers to invest in adequate cooling and/or holding facilities. This would result in a better raw product, which in turn would result in a more uniform butter. J. J. Janzen

CHEESE

441. Cheese manufacture. D. M. Greer and J. L. Zink (assignors to Armour and Company). U. S. Patent 2,709,656. 1 claim. May 31, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 5: 663. 1955.

A cheese making process is described which makes use of a cylindrical chamber which rotates at a variable speed on its horizontal axis. The coagulated curd is cut, drained, and dry cured in the device. R. Whitaker

442. Processing innovations create better cheeses. A. V. GEMMILL, SR. Food Eng., **27**, 4: 82. 1955.

Several modifications have been introduced into the manufacture of Mozarella cheese at the P. Cambardella & Son Cheese Corp. plant at New Haven, Conn. The basic changes involve: 1. Use of a special slow-acting starter which produces a curd that does not require milling and imparts improved flavor and texture characteristics. 2. A special "quick-cure" of the ½ lb. balls of curd in a refrigerated brine. 3. Vacuum packaging of the cheese in plastic film. As a result of these changes, shelf-life of the product has been more than doubled. The cheese has improved melting characteristics and better flavor. Sales have increased and the Company has expanded its line of Italian cheeses.
T. J. Claydon

443. Pickling of ewes milk Danni cheese. I. SIRRY and S. D. RAKSHY. Indian J. Dairy Sci., **7**, 64. 1954.

A description of the pickling process of white soft cheese from ewes milk is given. Data are presented on cheese yield, development of acidity in cheese and whey, and scores of the pickled cheese. W. C. van der Zant

444. Studies on the indigenous method of Chhana-making. I. The influence of the conditions of coagulation and the type of milk on the production of Chhana. S. DE and S. C. RAY. Indian J. Dairy Sci., **7**, 113. 1954.

Chhana is an acid-coagulated milk product and is used as the base and filler for Indian milk-sweets. In Chhana-making milk is brought to the boiling point and then the coagulating soln. (citric or lactic acid) is added. The coagulated material is poured into a muslin bag to drain the whey. The coagulum (Chhana) then is used for the preparation of Rosogolla and Sandesh (sweets). A high quality product was obtained under the following conditions, *(a)* acidity of coagulant 1-2%, *(b)* pH of coagulation 5.4, *(c)* heat-treatment 30 sec. at 180° F., *(d)* fat % of milk 4-4.5, *(e)* s.n.f./fat of about 2.0. Buffalo milk was not suitable for Chhana making. W. C. van der Zant

CONDENSED AND DRIED MILKS; BY-PRODUCTS

445. More profit from powdered milk. ANON. Milk Prod. J., **46**, 5: 18. 1955.

The installation of new spray-drying equipment for making powdered milk has resulted in a 240% increase in net earnings for the last 6 mo. of 1954 for this Minnesota Cooperative Creamery.

The equipment installed was a 7,400 lb. per hr. double effect evaporator together with a 500 lb. per hr. spray drier. The rated capacity is 500 lb. of powder per hr., in operation the production runs from 675 to 700 lb. per hr.

Skimmilk is charged into the evaporator from preheaters. Concentration is accomplished in two stages, namely, 160° F. and 20.27 in. vacuum and 104° F. and 27.75 in. vacuum. The product is discharged into the spray drier at 40% solids.

The concentrated milk is jet-sprayed into the vortex of a 325° F. hot-air blast where it is flash-dried. The powder drops to the bottom of the spray dryer and a ribbon-screw conveyer removes the powder to a sacking device. The end product contains 3.5% moisture.

Costs, including labor, amortization of equipment, and all other costs excepting the cost of the raw milk, for reducing skim fluid to powder amount to less than 2¢ per lb. The cost of steam is less than ½¢ per lb. J. J. Janzen

446. Een methode voor het afmeten van pappen en vlaas ten behoeve van de butyrometrische vetbepaling (Method for measuring viscous milk products, when carrying out the butyrometric determination of the fat content). J. B. S. BRUS and J. C. T. VAN DEN BERG. Neth. Milk Dairy J., **9**, 42. 1955.

A method is described for measuring viscous samples with the aid of a syringe directly into the butyrometer. A detailed description of the syringe, test procedure and correction factors is presented. W. C. van der Zant

447. Milk treatment process. D. D. PEEBLES and P. D. CLARY, JR. (assignors to Western Condensing Co.). U. S. Patent 2,710,808. 2 claims. June 14, 1955. Offic. Gaz. U. S. Pat. Office, **695**, 2: 272. 1955.

Concentrated skimmilk containing 35-55% solids is mixed with 5-25% of a water-miscible solvent to form a thin-bodied liquid. Lactose is allowed to crystallize at 32-50° F. The temperature is then raised to 50-65° F. and again cooled. An additional amount of lactose crystallizes at 18-32° F. The lactose is then removed, thus yielding a high protein product in which the protein is uncoagulated and is fully dispersed. R. Whitaker

448. Process for the flocculation of colloids in Cheddar cheese whey. L. TUMERMAN, H. SILVESTRI, and C. BURKE (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,708,166. 14 claims. May 10, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 2: 251. 1955.

The colloidal proteins of Cheddar cheese whey are precipitated by heating the whey in the presence of not over 1% sulfur dioxide and at a pH between 3.5 to 4.5. The whey may be mixed with some acid coagulated or casein whey. R. Whitaker

DAIRY BACTERIOLOGY

449. Glutamine synthesis by Micrococcus pyogenes var. aureus. B. A. FRY, School of Biochem., Univ. of Cambridge. Biochem. J., **59**, 4: 579. 1955.

Cell-free extracts of *M. pyogenes* var. *aureus* and a number of other bacteria catalyze the synthesis of glutamylhydroxamic acid from glu-

tamic acid and hydroxylamine in the presence of ATP and magnesium ions. When hydroxylamine is replaced by ammonia an amide identified as glutamine is synthesized. These syntheses are accompanied by appearance of an equivalent amount of phosphate. The enzyme system in the cell-free extract is activated by optimum concentrations of manganous ions as effectively as by magnesium ions. The synthesis is inhibited by calcium ions, sodium fluoride, adenosine diphosphate, para-chloromercuribenzoate and methionine sulfoxide. Sodium fluoride is more effective in the presence of magnesium ions than in the presence of manganous ions. R. Jenness

450. The characteristics of Lactobacillus plantarum, L. helveticus, and L. casei. D. M. Wheater, NIRD, Univ. of Reading. J. Gen. Microbiol., **12**, 1: 133. 1955.

One hundred fifty-two strains of lactobacilli of this group were studied by means of carbohydrate fermentation, growth temperature tolerance, ability to grow in the presence of 4% bile salt, the Voges-ProsKauer reaction and the ability to clot yeast-glucose-litmus milk. On the basis of these tests, three groups of organisms were observed. Serologically, all strains of *L. plantarum* grouped together and a majority of strains of *L. helveticus* behaved similarly. However, the *L. casei* cultures classified together on the basis of physiological tests were divided into two groups on the basis of the serological tests. J. J. Jezeski

451. The characteristics of Lactobacillus acidophilus and Lactobacillus bulgaricus. D. M. Wheater, NIRD, Univ. of Reading. J. Gen. Microbiol., **12**, 1: 123. 1955.

Ninety-four strains of lactobacilli were studied by means of sugar fermentations and tolerance to sodium chloride and sodium tauroglycocholate. Three groups of organisms were observed as a result of these studies. Cultures of *L. acidophilus* fermented several carbohydrates and grew in 2% sodium chloride or sodium tauroglycocholate; whereas strains of *L. bulgaricus* did not ferment the same carbohydrates and failed to grow in the presence of the latter two substances. A third intermediate group with characteristics falling somewhere in between these two extremes was likewise observed. There did appear, however, to be some variability in the serological classification of these organisms included in this study, at least of those classified as *L. bulgaricus*. J. J. Jezeski

452. A serological classification of lactobacilli. M. E. Sharp, NIRD, Univ. of Reading. J. Gen. Microbiol., **12**, 1: 107. 1955.

Immune rabbit sera were prepared against some typical strains of lactobacilli, and crude HCl extracts of a large variety of isolated lactobacillus cultures were tested against the sera by means of a precipitin test. A collection of 442 strains of lactobacilli isolated from a variety of sources and which included representatives of all typical species were studied. Seventy % of these cultures fell into six groups and one subgroup. The serological classification of lactobacilli so obtained was in reasonable agreement with a classification based on the physiological characteristics of these organisms; however, the study was complicated by the frequent appearance of non-specific reactions. These difficulties could apparently be surmounted in many cases. J. J. Jezeski

453. Relationships between viable saccharolytic bacteria in rumen and abomasum of the young calf and kid. S. O. Mann, and A. E. Oxford, Rowett Research Institute, Bucksburn, Aberdeenshire. J. Gen. Microbiol., **12**, 1: 140. 1955.

Large numbers of lactobacilli of both homo- and heterofermentative types were observed in the abomasa of three young calves and two kids fed on milk diets. A heterofermentative *L. fermenti* was observed in both the abomasum and rumen of one calf. Organisms resembling *L. acidophilus* were found in a calf as well as in the abomasa of the kids. An atypical variety of amylolytic streptococcus was isolated from the rumen and abomasum of a young calf and differed from the typical *S. bovis* of the adult rumen. J. J. Jezeski

454. Über die Keimauslese in Milch bei den verschiedenen Erhitzungsverfahren (Effect of various heating methods on the bacterial content of milk). W. Kundrat, Wihenstephan, Germany. Milchwissenschaft, **10**, 79. 1955.

A review of literature dealing with the destruction of bacteria during pasteurization by different methods, the destruction of pathogenic and heat resistant organisms, and the sources of contamination of raw milk is given. J. Tobias

455. De thermoresistentie van enige bacteriophagen geisoleerd uit zuursels en yoghurtculturen (The thermoresistance of some bacteriophages isolated from starters and yoghurt cultures.). K. Stolk. Neth. Milk Dairy J., **9**, 37. 1955.

The thermoresistance of 16 bacteriophages, 9 from lactic starters and 7 from yoghurt cultures, was determined. The bacteriophages from the starters showed great differences in their thermoresistance. Some were destroyed after heating at 75° C. for 10 sec., others were inactivated at 90-95° C. (20-10 sec.). All phages from the yoghurt cultures were inactivated after heating at 85° C. for 10 sec. In order to free milk from bacteriophage it is recommended to heat the milk until the peroxidase reaction is negative. W. C. van der Zant

456. Over het aantal niet thermoresistente melkzuurstreptococcen in gepasteuriseerde melk bepaad direct na het bottelen (The

initial concentration of S. lactis and S. cremoris in HTST pasteurized milk). T. E. GALESLOOT. Neth. Milk Dairy J., 9, 30. 1955.

The number of non-thermodurie streptococci in pasteurized milk after bottling was very small. The keeping quality (at 20° C., 48 hr.) of the milk was poor when these organisms were present in 1-5 ml. of the sample.

W. C. van der Zant

457. Effect of feeding curds, sulphaguanidine and paraaminobenzoic acid on the coliform organisms and biosynthesis of thiamine. S. BALAKRISHNAN and R. RAJAGOPALAN. Indian J. Dairy Sci., 7, 126. 1954.

Sulphaguanidine suppressed almost completely the biosynthesis of thiamine and also the coliform bacteria in the feces. Sulphaguanidine together with PABA did not suppress thiamine synthesis nor reduce the number of coliform organisms. The inclusion of curd increased thiamine synthesis and also the number of coliforms in the intestine. The synthesis of thiamine and the number of coliforms were not significantly affected when curd was fed together with sulphaguanidine.

W. C. van der Zant

DAIRY CHEMISTRY

458. Electrophoretische Studien an Milchproteinen. Die papierelectrophoretische Trennung der Eisweisstoffe der Kumilch und der dazugehörigen Molke (Study of milk proteins by means of electrophoresis. Separation of albumins of cow's milk and the pertinent whey by paper electrophoresis.). K. E. SCHULTE and F. MÜLLER, Pharm. Inst. der Freien Univ., Berlin, Germany. Milchwissenschaft, 10, 90. 1955.

The proteins of milk were shown to contain 7 components, and those of whey 8 components. The characteristic features of each of the fractions are given and a new nomenclature is suggested for 7 of the protein fractions found in whey.

J. Tobias

459. Quantitative determination of glucose and galactose. H. FISHER, R. G. HANSEN, and H. W. NORTON, Univ. of Ill., Urbana. Analyt. Chem., 27, 5: 857. 1955.

The simultaneous quantitative determination of glucose and galactose in biological materials is of importance in the study of galactose and lactose metabolism in various animals. A rapid and sensitive method, based upon the orcinol procedure of Brückner, is used for the simultaneous estimation of glucose and galactose in mixtures. Results are usually within 0.03 mg. for glucose and 0.01 mg. for galactose in the concentration range of 0 to 0.150 mg. for each sugar.

The procedure is most useful for quantitative determination of both glucose and galactose in blood but it can probably be used also in other materials.

B. H. Webb

460. Qualitative determination of amino acids in protein hydrolyzates by circular paper chromatography. I. OREskes and A. SAIFER, Dept. of Phys. Chem., Jewish Chronic Disease Hosp., Brooklyn 3, N. Y. Analyt. Chem., 27, 5: 854. 1955.

The circular chromatographic technique described in this paper will permit identification of all of the 19 amino acids usually found in protein hydrolyzates, except for leucines, which are determined as a pair. This is achieved by running two separate chromatograms for each hydrolyzate with phenol and butanol-acetic acid as the two developing solvents. After acetone washing to remove phenol and drying, each chromatogram is cut into halves, one half being dipped into ninhydrin and the other in isatin as the two coloring reagents. R_f values compare favorably with those obtained by other investigators using buffered filter papers. The small size of apparatus and the speed and simplicity of the method make it very useful for routine analysis of amino acids.

B. H. Webb

461. Food. J. R. MATCHETT and H. W. VON LOESECKE, Wash. URB, ARS, USDA, Washington, D. C. Analyt. Chem., 27, 4: 623. 1955.

This review covers papers published during the last 2 years and includes the following subjects: moisture, proteins and amino acids, metallic ions, fats and oils, enzymes, carbohydrates, vitamins, acids, color and taste, insecticide residues, contamination and spoilage, and miscellaneous. 313 references are cited.

B. H. Webb

462. Lactic acid purification by extraction. R. B. WEISER and C. J. GEANKOPLIS, Ohio State Univ., Columbus. Ind. Eng. Chem., 47, 4: 858. 1955.

This program was undertaken to investigate thoroughly liquid-liquid extraction as a possible purification method for crude lactic acid. A solvent search was first carried out to determine the best selective solvent for lactic acid in preference to the usual impurities found in the crude solution. Then, using the best selective solvent, further processing problems were investigated on a laboratory scale.

On the basis of the data obtained, isoamyl alcohol appeared to be the best selective solvent. A complete ternary phase and equilibrium diagram was obtained for the water-lactic acid-isoamyl alcohol system. Steam distillation was used as the solvent recovery method. These results indicate that the process has some possibilities for industrial application, but further larger scale work is required for a complete evaluation.

B. H. Webb

463. Een methode voor de bepaling van het kopergehalte van melk en melk poeder geschikt voor serie-onderzoek (A routine method for the determination of copper in milk and in dried milk). B. M. KROL and P. C. DEN HERDER. Neth. Milk Dairy J., 9, 56. 1955.

This extraction method consists of adding alcoholic HCl to the milk to precipitate the milk protein and to dissolve the copper. After elimination of the proteins the copper in the clear filtrate is determined colorimetrically with diethyl-dithio-carbamate. A detailed description of the method and reagents is presented.
W. C. van der Zant

464. Microbiological assay of essential amino acids in the milk of Indian buffalo. N. V. Joshi and H. Raj. Indian J. Dairy Sci., 7, 139. 1954.

The contents of tryptophan, leucine, isoleucine, valine, lysine, phenylalanine, methionine, threonine, histidine, arginine, and cystine in buffalo milk were approx. the same as those reported for cows' milk. W. C. van der Zant

465. Calculation of total solids in milk of cows in Israel by Richmond's formula. J. Babad, A. Pinsky, and N. Sharon. Indian J. Dairy Sci., 7, 98. 1954.

The % total solids calculated with Richmond's formula was usually higher than that obtained by evaporating to constant weight. The milk samples were collected in an area where subtropical conditions prevail.
W. C. van der Zant

466. Ultra-geluidsgolven en de mogelyke toepassing ervan in de zuivelindustry (Ultrasonic waves and the prospects of their application in the dairy industry). T. E. Galesloot. Neth. Milk Dairy J., 9, 88. 1955.

A review of literature on the application of ultra-sonic waves in dairy research and industry is presented. In the author's opinion only few possibilities exist at the present time of applying ultra-sonic waves successfully in the Dairy Industry. (70 references.)
W. C. van der Zant

467. Studies on the heterogeneity of crystallized β-lactoglobulin. A. G. Ogston and J. M. A. Tilley, Dept. of Biochem., Univ. of Oxford. Biochem. J., **59**, 4: 644. 1955.

The electrophoretic migration of β-lactoglobulin was studied under various conditions of concentration, pH, and temperature. The data obtained indicate the presence of two very similar chemical species in proportions which may vary from one sample to another and which can be separated from each other only with great difficulty. It appears that one of these species associates reversibly to form a complex which migrates and sediments more rapidly than the simple material. The sedimentation data suggest that this is a dimer. The association of this species is strongly dependent upon temperature and pH. The heterogeneity of β-lactoglobulin was confirmed by solubility measurements. R. Jenness

468. Sulphydryl groups in haemoglobins. V. M. Ingram, Cavendish Lab., Univ. of Cambridge. Biochem. J., **59**, 4: 653. 1955.

Amperometric titrations with silver nitrate and mercuric chloride were used to determine the numbers of available sulfhydryl groups in native and sodium dodecyl sulfate denatured hemoglobins from horse, ox, man, and sheep blood. Native horse, ox, and human hemoglobins were found to contain four sulfhydryl groups as two pairs of closely neighboring groups per molecule. Native sheep hemoglobin contained eight sulfhydryl groups as four such pairs. Upon denaturation with sodium dodecylsulfate, horse hemoglobin exhibited six titratable sulfhydryl groups; whereas with the same treatment, human hemoglobin exhibited eight sulfhydryl groups. R. Jenness

469. Separation of oestrogens in urinary extracts by partition chromatography. W. S. Bauld, Dept. of Biochem., Univ. of Edinburgh. Biochem. J., **59**, 2: 294. 1955.

A method is described on the separation and purification of oestriol, oestrone, and oestradiol-17 β in extracts of hydrolyzed urine by means of partition chromatography on celite columns. The principal factors affecting the elution of oestrogens from the partition chromatograms are described in full. R. Jenness

470. The estimation of unsaturated fatty acids by reversed-phase partition chromatography. W. M. L. Crombie, R. Comber, and S. G. Boatman, Dept. of Botany, Univ. of Southampton. Biochem. J., **59**, 2: 309. 1955.

The separation of representative unsaturated acids was investigated on a reversed-phase column of water-repellent keiselguhr impregnated with liquid paraffin employing acetone-water mixtures for elution. A method is presented for analysis of mg. quantities of fatty acid mixtures containing both saturated and unsaturated acids. The common unsaturated fatty acids are separable from each other by this technique but not from certain saturated fatty acids which are eluted at similar rates. The latter can be estimated by a second chromatogram following oxidation by alkaline permanganate to remove the unsaturated material. The amounts of unsaturated fatty acids can then be determined by difference. The method was tested on standard fatty acid mixtures and on fatty acids from a number of natural oils. It gave results in good agreement with those obtained by other techniques. R. Jenness

471. The occurrence of a cyanocobalamin-binding protein in milk and the isolation of a cyanocobalamin-protein complex from sow's milk. M. E. Gregory and E. S. Holdsworth, NIRD, Univ. of Reading. Biochem. J., **59**, 2: 329. 1955.

It was demonstrated by an ultrafiltration technique that milks of different animal species have the property of combining with added cyanocobalamin (vitamin B_{12}). The amount of vitamin bound per ml. of milk was: pig—0.24 μg.; rat—0.13 μg.; woman—0.08 μg.; goat—

0.0027 μg.; and cow—0.0005 μg. The whey proteins of each of the milks were separated by electrophoresis on paper and the position of the proteins on the paper compared with the position in which the vitamin B_{12} occurred. The latter was determined either by microbiological analysis or by radioautography using Co^{60}-labeled vitamin B_{12}. The vitamin B_{12} did not move at the speed of any of the major proteins of the whey and hence it was concluded that the combining factors is not one of the major proteins. Vitamin B_{12}-protein complex had the same mobility in each of the milks tested. A pure vitamin B_{12}-protein complex was isolated from sow's milk whey by fractional precipitation with ammonium sulfate, separation from the bulk of the proteins by continuous electrophoresis on paper and finally fractional precipitation with isopropanol. R. Jenness

472. Some properties of the cyanocobalamin-protein complex from sow's milk, and the mode of linkage of cyanocobalamin with protein. M. E. Gregory and E. S. Holdsworth, NIRD, Univ. of Reading. Biochem. J., **59**, 2: 335. 1955.

The cyanocobalamin (vitamin B_{12})-protein complex isolated from sow's milk whey was analyzed. It was found to contain 16.1% nitrogen, 7% carbohydrate, and 9% hexosamine. Its amino acid composition was typical of proteins except for a high tyrosine content (17.6%). The absorption curve of the complex in aqueous solution exhibited peaks at 278 mμ (due to protein) and at 362, 410, 520, and 550 mμ (due to vitamin B_{12}). The vitamin B_{12} content of the complex is 23.6 μg. per mg. calculated on the absorption curve and 22.4 μg per mg. measured by *E. coli*. From these figures and assuming that one molecule of protein combines with one molecule of vitamin B_{12}, a molecular weight of 55,000 was calculated for the protein. The use of specific reagents to block different groups on the protein molecule showed that either a phenolic or an amino group on the protein may be concerned in its combination with the vitamin. R. Jenness

473. An apparatus for continuous electrophoresis on paper. E. S. Holdsworth, NIRD, Univ. of Reading. Biochem. J., **59**, 2: 340. 1955.

An apparatus is described for continuous electrophoresis on paper. This includes a flowing electrode system which enables the apparatus to be operated for long periods without pH change on the paper. The paper is cooled so that the technique may be applied to heat labile materials such as enzymes. The apparatus may be used for the continuous separation of mixtures in amounts up to 4 ml. of a 5% solution in 24 hours. R. Jenness

474. The branched-chain fatty acids of butterfat. 6. Further investigations on the C_{15} saturated acids. F. B. Shorland, T. Gerson, and R. P. Hansen, Dept. of Sci. and Ind. Research, Wellington, N. Z. Biochem. J., **59**, 2: 350. 1955.

Two C_{15} branched-chain acids (*)-12-methyltetradecanoic acid and 13-methyltetradecanoic acid, together with the straight-chain odd-numbered acid *n*-pentadecanoic acid, were isolated from butterfat by methods not involving hydrogenation. The amounts of these acids expressed as percentage of the total fatty acids in butterfat were, respectively, 0.43, 0.37, and 0.82%. R. Jenness

475. Apparatus and technique for two-dimensional paper ionophoresis. T. H. Mead, Dept. of Pathology, Univ. of Cape Town. Biochem. J., **59**, 4: 534. 1955.

A new apparatus and a technique for two-dimension paper ionophoresis of amino acids are described. In this method the pH of the buffer solutions in the paper is changed after ionophoresis in one dimension by exposure to the vapor from a solution of ammonia at constant partial pressure. A method is described for tracing movements of electrolyte in the paper with a sugar-spot grid applied to the paper. Irregularities in the movement of fluid over the paper were reduced by adjustment of the cooling. The following amino acids were separated qualitatively by this apparatus and technique: glutamic and aspartic acids, cysteine, glycine, alanine, valine, leucine, phenylalanine, serine, threonine, tyrosine, hydroxyproline, proline, histidine, lysine, and arginine. Leucine and isoleucine were not separated. An experiment with this technique can be completed in nine hours. R. Jenness

476. Separation of neutral proteins on ion-exchange resins. N. K. Boardman and S. M. Partridge, Univ. of Cambridge. Biochem. J., **59**, 4: 543. 1955.

The separation of proteins by chromatography on the ion-exchange resin IRC-50 was studied. Changes in pH and cation concentration of the buffer affected the adsorption of cytochrome c much differently than the adsorption of lysine. Lysine was desorbed as the pH fell below 5 due to the suppression of the ionization of the carboxyl groups of the resin but the adsorption of cytochrome c was greatly increased under acidic conditions. It is suggested that this adsorption is due to the large increase in secondary short-range forces. By elution chromatography on columns of IRC-50 sheep fetal carbon monoxide hemoglobin could be separated from sheep maternal CO hemoglobin and from bovine CO hemoglobin. Bovine CO hemoglobin could be separated from bovine methemoglobin. These separations were sharply dependent upon pH and on sodium ion concentrations in the eluting buffer. To obtain good yields it was necessary to work in temperatures near 0° C. and use freshly prepared proteins. Bovine CO hemoglobin was not altered by pass-

age through the column. It could be readily crystallized. R. Jenness

477. The determination of calcium in serum by flame photometry. R. W. R. Baker, Dept. of Chemical Pathology, Guy's Hospital Medical School, London, S.E. 1. Biochem. J., **59**, 4: 566. 1955.

An internal standard flame photometer for estimating calcium, sodium, and potassium in serum is described together with a method which involves only dilution of the serum sample.

R. Jenness

478. Interference by amino acids in the estimation of sugars by reductometric methods. R. E. Strange, F. A. Dark, and A. G. Ness, Microbiol. Research Dept., Porton, Wiltshire. Biochem. J., **59**, 1: 172. 1955.

The interference by amino acids in the estimation of glucose by reductometric methods using copper and ferricyanide was investigated. Copper was reduced by cystine, tyrosine, and tryptophan, but in general was less affected by amino acids than was ferricyanide. A casein hydrolysate alone had little effect on copper but recoveries of glucose from samples containing casein hydrolysate were incorrect, some being high and some low. The reducing power of a solution of acid-hydrolyzed casein for ferricyanide was not substantially lowered by precipitation with barium, cadmium, or mercury. A method is described for removing the interfering amino acids by treatment with a synthetic cation exchange resin IR-20 (H) with the anionic resin IR-4B (OH) to avoid excess acidity. This technique should be especially applicable to such materials as protein hydrolysate or bacteriological culture media where the interference from amino acids may be very large.

R. Jenness

DAIRY ENGINEERING

479. Refrigeration in the dairy industry. E. E. Bergstrom, Safeway Stores, Inc., San Francisco, Calif. Ind. Refrig., **128**, 5: 32. 1955.

All phases of refrigeration application in the dairy industry from producer with bulk tank storage, through processing, and delivery of finished products by refrigerated trucks is covered. Of particular interest is a schematic drawing of the refrigeration installation for a recently constructed dual milk and ice cream plant. Accompanying this is a mock-up of the plant with description of processing rooms whereby the reader can correlate refrigeration to equipment. L. M. Dorsey

480. Heat exchanger. R. R. Graves and E. K. Kuhles (assignors to The Graves-Stambaugh Corp.). U. S. Patent 2,706,619. 6 claims. Apr. 19, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 3: 357. 1955.

An enclosed sleeve-in-casing type of heater for rapidly raising the temp. of milk. The heater is of sanitary design and relatively little gasket area is exposed to the product.

R. Whitaker

481. Heat exchanger. R. R. Graves (assignor to The Graves-Stambaugh Corp.). U. S. Patent 2,706,620. 2 claims. Apr. 19, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 3: 357. 1955.

Similar to heater mentioned in Abstr. ‡480.

R. Whitaker

482. Heater for multiple effect evaporators. W. E. Hesler (assignor to Whiting Corp.). U. S. Patent 2,707,022. 3 claims. Apr. 26, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 4: 463. 1955.

The tube chest between the two stages of a double effect evaporator is sectioned off into two compartments, each equipped with tubes and so arranged to increase the efficiency of the heating of the product in its passage through the tube chest. R. Whitaker

483. Pasteurization of heat sensitive liquids. E. Nanz (assignor to Societe pour l'Equipement des Industries Laitieres et Agricoles (S.E.I.L.A.) and Walter Joseph Sidler). U. S. Patent 2,708,167. 8 claims. May 10, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 2: 251. 1955.

Milk and other heat sensitive liquids are heated in not over 2 secs. in this pasteurizer, consisting of two heating compartments. The first compartment upstream is under slight negative pressure, whereas the second is at atmospheric. Means of rapid continuous cooling are provided. R. Whitaker

484. HTST cream pasteurizer. Anon. Milk Prod. J., **46**, 6: 14. 1955.

This article describes the use of a completely automatic HTST cream pasteurizing system that has recently been installed in a dairy plant. Whole milk is heated to 80° F. in a preheater, separated, and the cream then routed to a balance tank, vacuumizer and HTST pasteurizer.

The cream is pasteurized at 190° F., part of it is by-passed to the balance tank where it heats the 80° F. cream to 160° F. prior to vacuum treatment. The vacuumizer works best on cream at 160° F.

The cream is cooled, by passing over a plate heater, to 42°-45° F. The process has proven valuable from the standpoint of quality and time and space-saving features.

J. J. Janzen

DAIRY PLANT MANAGEMENT AND ECONOMICS

485. How to get an accurate physical inventory in the shortest possible time. H. Young, Better Packaging Advisory Council. Ice Cream Rev., **38**, 10: 68. 1955.

A simple system for taking the complete physical inventory of a plant is described. The system provides for a high degree of accuracy

since the count on each item is checked independently by each of two counting teams. Responsibility for errors is fixed and the actual counting work is made relatively easy for the participants. W. J. Caulfield

486. Unit operations in chemical engineering. Ind. Eng. Chem., **47**, 3: 501. 1955.

The sections of this review have been prepared by many authors. Papers of interest in dairy product processing and research include those on Centrifugation, Crystallization, Drying, Evaporation, Filtration, Ion Exchange, and Materials Handling. B. H. Webb

487. Team cooperation in the bulk story. D. MERLIN. Milk Prod. J., **46**, 6: 16. 1955.

The problems and advantages of bulk pick-up are discussed from the standpoint of the producer, the hauler, and the milk dealer.

J. J. Janzen

488. Bulk pick-up under lab conditions. G. H. WATROUS, JR., J. E NICHOLAS, and R. W. DECKER, Penn. State Univ. Milk Plant Monthly, **44**, 5: 45. 1955.

Basically there are two major types of bulk milk vats. In the direct expansion type, the milk is cooled directly by contact with a refrigerated plate utilizing freon as the cooling medium. The ice bank cooler builds up an ice bank and the ice in turn is used for cooling water and chilled water used as the cooling medium. Laboratory experiments with two vats of each type were conducted and data are presented showing: (1) Suction and discharge pressure of the compressor, (2) Wattage, voltage, and amperage during operations, (3) Kilowatt hours required by the compressor, agitator, and water circulator, (4) Cooling rates, (5) Total operating time of the unit, and (6) Quantities of water supplies to condensers when water was used. C. J. Babcock

489. Bulk pick-up one hundred per cent. H. E. BIRDSALL, Twin City Producers Assoc. Milk Plant Monthly, **44**, 5: 41. 1955.

The 100% conversion from cans to bulk tanks would offer enough economies to permit producers to receive as much for their milk as they do in cans. Furthermore, the combined savings of the producer and plant would pay for all the tanks within a reasonable time. The savings to the producer would be elimination of cans, 1.4¢; elimination of dumping loss, 2.5¢; consolidation of hauling, 5.0¢—a total of 8.9¢ per cwt.

The producers, acting as a group, would also bring about some savings to the Association which, in turn, is the producer. These are: 2.22¢ per cwt. paid to dealers for receiving the milk and washing the cans, and 5.57¢ brought about by closing intakes in the receiving plants owned by the Association. A total group savings of 7.79¢ per cwt. which would be placed in a tank equity fund, and would not affect the returns to the producer because at present it is being expended in handling the milk in cans. The producer would also loan back to the Association 5¢ of his 8.9¢ savings, making a total of 12.79¢ which would go into the tank equity fund. A total expense of 1.49¢ per cwt. would come out of this fund leaving 10.84¢ for every 100 lb. of milk each member produced to allocate back to all producers on a patronage basis at the end of each year. On this basis, the entire investment for all tanks would be recovered by the Association within 9½ years or less. Producers would, therefore, earn titles to their tanks in from four to 20 years.

C. J. Babcock

490. Bulk pick-up and the hauler. J. E. EDMONSON and J. H. GHOLSON, Univ. of Mo., Columbia. Milk Plant Monthly, **44**, 5: 26. 1955.

One of the problems recognized by the dairy industry is the additional training needed by bulk tank haulers. A short course given at the Univ. of Mo. qualifies haulers to properly check and identify abnormal flavors and odors in milk, to determine accurately the volume of milk, to sample milk correctly for butterfat and other laboratory tests, and to suggest good sanitary practice for milk production.

C. J. Babcock

491. Bulk pick-up financing plan. C. O. DAVIS, JR. Milk Plant Monthly, **44**, 5: 23. 1955.

The cost of a farm tank plus the necessary milkhouse improvements usually runs from $2,000 up. The basic points of the Chicago Pure Milk Association's financing plan are: (1) Plant operator agrees to pay 15¢ per cwt. premium for tank milk. Producer will pay 5¢ per cwt., (2) Pure Milk Association arranges for local financing at not more than 6% per annum to cover tank cost and installation, (3) Local dealers of approved tanks arrange a showing and producers select the tanks they want. Approved tanks are those of manufacturers agreeing to P. M. A.'s standards, which include a 10-year guarantee on the bank and five-year guarantee on compressors. Other requirements include compliance with 3-A standards and the regulations of the Chicago Board of Health. Specific standards cover level check devices, thermometers, covers, legs, removal methods, calibration, motors, and agitation, and (4) Tank dealers prepare conditional sales contract to be signed by producers, setting up five-year loan to be paid from the 20¢ per cwt. described in "1" above. C. J. Babcock

492. Bulk pick-up electrical costs. C. W. HALL, Mich. State Coll., East Lansing. Milk Plant Monthly, **44**, 5: 20. 1955.

Tests on 19 bulk tanks in the field and five tanks in the laboratory under controlled condi-

tions were carried out to measure the field cost of operation for bulk milk tanks for every other day and every day pickup of milk, the cost of electrical energy for operation of bulk milk tanks when operating under laboratory conditions, the effect of various amounts of filling on operational costs, the seasonal cost of operation, the effect of milk house management, and the fixed cost of operation. The latter is presented graphically and tables are given showing the average cost of electrical energy for individual farm bulk tanks, and a comparison of laboratory and field operation. C. J. Babcock

493. Bulk pick-up and the sanitarian. D. B. WHITEHEAD, Miss. State Board of Health, Jackson. Milk Plant Monthly, **44**, 5: 18. 1955.

Wherever roads and other circumstances permit and a specific policy and procedure is rigidly followed the bulk farm pick-up system materially improves both the physical and the bacteriological quality of any given milk supply. Three factors to consider are equipment and equipment design, installation and procedures, and the legal aspects of sampling. It is pointed out that proper cover design, better agitation methods, position and construction of outlet valves, refrigerating capacity, and overall size, all need to be considered. Producers and haulers must be thoroughly instructed in cleaning and sanitizing. Sufficient work space around the tank and proper hose handling are necessary, and haulers must be trained. There must be close inspection of all equipment, samples must be taken from the farm tank and sanitarians must work out practical systems for taking them. C. J. Babcock

494. Bulk pick-up gains momentum. C. O. DAVIS, JR. Milk Plant Monthly, **44**, 5: 15. 1955.

It is estimated that by July 1955, there will be 25,000 tanks in use and in less than 10 years 75% of the total milk supply in the U. S. will be in farm tanks. The principal problems in switching to bulk pick-up are financing, lack of uniform standards, off-flavors, need for trained haulers, and providing for small producers. C. J. Babcock

495. The effect of methods of paying farmers for milk on seasonality of production in selected southern markets. W. W. MOFFETT, JR. *et al.*, Miss. Agr. Expt. Sta., State College. Sou. Coop. Series, Bull. 37. 1954.

The objectives of this study were to determine the relationship between methods used in the South for paying farmers for fluid milk and seasonality of production, and to secure information regarding the payment plans employed in the South by producers, dealers, and regulatory agencies. Seventeen milk markets located in 7 states were investigated.

Pricing plans were classified as base-surplus, use classification, combination base-surplus and use-classification, and flat price plans. The base-surplus, use-classification, and combination price plans were used to purchase 36, 35, and 26%, respectively, of the total milk purchased. Only 3% of the total milk is purchased under the flat price plan. Price incentive was greatest in those markets using a combination of base-surplus and use-classification plans. Producers whose supply closely followed the seasonal demand pattern received 43¢ more per cwt. than highly seasonal producers under the combination plan, 32¢ more under the base-surplus plan, and 9¢ more under the use-classification plan. R. W. Hunt

496. North Dakota's dairy marketing problems in historical perspective. L. A. FOURT and G. A. KRISTJANSON, N. D. Agr. Expt. Sta., Fargo. Bull. 393. 1954.

The purpose of this study is to describe and explain the development of conditions and practices in the marketing of dairy products since N. D. became a state in 1889. The marketing of butterfat is the primary portion of the study, due to its relative importance within the dairy industry of this state.

R. W. Hunt

497. Southern agriculture and world trade. J. N. EFFERSON, Dept. of Agr. Econ., La. State Univ., Baton Rouge. Mimeographed Cir. 159. 1954.

This circular describes the complexity of world trade and how it affects an individual citizen of the U. S. Since World War II, this country has been exporting about 16 billion dollars worth of goods per yr. and importing only 11 billion. The 5 billion dollar deficit has been cared for by either gifts or loans provided by the U. S. government or by the foreign countries reducing the reserves of purchasing power. A lengthy discussion of possible solutions to this unequal trade situation is presented. This publication is written in a manner which can be easily understood by all interested persons. R. W. Hunt

498. Reducing fatigue in the milk products plant. R. J. CARSON. Milk Prod. J., **46**, 5: 28. 1955.

Fatigue causes dissatisfaction with working conditions. Dissatisfaction can be alleviated and the job and work surroundings made more pleasant by a few simple precautions.

The author lists and briefly discusses the following seven point plan, which should help in alleviating fatigue: (1) poor lighting should be improved, (2) good ventilation, (3) avoid excessive noise, (4) proper nourishment, (5) regulating work hours, (6) suitably staggered rest periods, and (7) use of efficient work methods. J. J. Janzen

499. Recombined milk for dairy products in Caribbean, Central America. D. R. STROBEL, USDA. Milk Prod. J., **46**, 5: 23. 1955.

There is a potential long-range market abroad

for U. S. NFDMS and butter-oil (anhydrous milk fat) for use in the production of recombined milk. There are extensive possibilities of developing this market in view of the need for recombined milk to supplement milk supplies in periods of short production in certain world areas, and to provide a permanent milk supply in others.

A study of the Caribbean and Central American countries has shown a per-capita consumption of milk below that usually accepted as necessary for a balanced diet. The problem is twofold: (1) provide a safe milk supply and (2) educate the inhabitants as to the nutritive value of milk.

The author reports on the Dominican Republic, Haiti, Cuba, Honduras, and Nicaragua. It is concluded that there is a market for recombined milk especially during the season of short supply, providing USDA carlot export prices apply. However, in Honduras, the production of recombined milk is not looked upon with favor. J. J. Janzen

500. Marketing dairy products as ghee and recombined milk. L. H. BURGWALD. Milk Prod. J., **46**, 6: 22. 1955.

A Foreign Agricultural Service survey was made recently in the Far East, the Mediterranean area and Europe to obtain certain specific information regarding the marketing of United States dairy products in those parts of the world.

Each of the areas visited, except the United Kingdom, poses a large potential market for recombined milk. However, before an effective demand can be created, pioneering, promotional, and educational work will be necessary. Lack of refrigeration in certain areas poses additional problems.

India, Pakistan, and Egypt are potential markets for ghee, which can readily be supplied by the United States dairy industry.

J. J. Janzen

FEEDS AND FEEDING

501. Effect of feeding iodinated casein on calcium and phosphorus metabolism in young heifers. G. K. MURTHY, N. N. DASTUR, and S. C. RAY. Indian J. Dairy Sci., 7, 93. 1954.

One of 2 groups of Sindhi and Gir heifers was fed 2 g. iodinated casein per 100 lb. body weight daily for 20 weeks. Except for a somewhat higher absorption coefficient for P, no significant differences were observed in the Ca and P metabolism. W. C. van der Zant

502. Yield and composition of corn forage as influenced by soil fertilization. K. E. HARSHBARGER, *et al.*, Ill. Agr. Expt. Sta., Urbana. Bull. 577. 1954.

Under the conditions of this 5-yr. trial, the application of fertilizers to soils for the production of corn forage resulted in: (1) gains in tonnage of forage, (2) little or no change in the proportion of forage which consisted of ears, (3) little change in protein content of the grain (shelled corn) except as influenced by season, (4) an enhanced protein content of leaf-stalk when the crop was grown on poor soil, and (5) little change in the crude-fiber content of the forage. R. W. Hunt

503. Effect of certain adsorbents and mineral mixtures on the availability of riboflavin and other B-vitamins in rations. C. H. HUNT, O. G. BENTLY, T. V. HERSHBERGER, and A. L. MOXON, Ohio Agr. Expt. Sta., Wooster. Bull. 748. 1954.

The question is often raised as to what effect mineral mixtures and fillers have on the over-all utilization of livestock feeds. The results reported in the study are based on the utilization of riboflavin, niacin, and pantothenic acid when fed to laboratory animals in the presence of vitamin carriers, minerals, and trace mineral mixtures.

Solutions of riboflavin when mixed with bone meal, limestone, dicalcium phosphate, and one brand of Fuller's earth were readily available for the growth of microorganisms and laboratory animals. Riboflavin was not available when fed in conjunction with Norite A, bone black, and two brands of Fuller's earth. When alfalfa meal and dried skimmilk were the sources of riboflavin, and fed at a level of 10% of the ration, along with bentonite, bone black, and soft clay rock phosphate, only bentonite interfered with the availability of riboflavin. Should the alfalfa meal and skimmilk constitute only 5% of the ration, the availability of riboflavin was reduced by all the additives. This would suggest that the deleterious effect of certain adsorbents may be overcome by supplying higher levels of vitamins in the feed. Two trace mineral mixtures were used. One contained fat which became rancid at room temperature. With the increase in the peroxide number of the fat, a definite loss of pantothenic acid was found. Both mixtures were satisfactory when the amounts of vitamin-B complex were increased and fat decreased in ration 2. Salts of the trace minerals, iron, manganese, copper, and cobalt individually or as a group in a simulated trace mineral mixture did not affect the availability of pantothenic acid.

R. W. Hunt

504. Animal feeding apparatus. T. ALBERS. U. S. Patent 2,706,581. 8 claims. Apr. 19, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 3: 347. 1955.

A feeding device, installed between 2 cows in adjacent stalls, consisting of a storage bin and gates, and chutes for delivering the feed to each animal. R. Whitaker

GENETICS AND BREEDING

505. A survey of the bacteria in the reproductive tract of dairy animals and their relationship to infertility. J. J. GUNTER, W. J.

COLLINS, J. OWEN, A. M. SORENSEN, J. W. SCALES, and J. A. ALFORD, Miss. Agr. Expt. Sta., State College. Am. J. Vet. Research, **16**, 59: 282. 1955.

Sterile swab samples were taken from the uterus or cervix of both normal and difficult breeding cows at successive heat periods. The samples were incubated in thioglycollate broth and on blood agar aerobically, anaerobically, and in 10% CO_2. This multiple isolation technique recovered organisms from many samples that would have rated sterile by a single procedure. Sterile samples were secured from 22% of the normal cows but none of the repeat breeders. Of 260 samples from 106 cows, 38% of 201 from 92 normal cows were sterile; but only 5% of 59 from 14 repeat breeder cows were sterile. The predominant types of bacteria among 400 cultures identified in detail were diphtheroids and micrococci. Most of the cultures from the difficult breeders contained potential pathogens. In the normal breeders, the strains of bacteria were predominantly saprophytic. The same isolations from the repeat breeding cows were obtained consistently at successive heat periods. These results suggest a connection between low-grade uterine infection and low fertility. E. W. Swanson

HERD MANAGEMENT

506. Is your dairy farm in balance? L. H. KELLER, F. M. DEFRIESE, and T. J. WHATLEY, Tenn. Agr. Expt. Sta., Knoxville. Farm Econ. Cir. 1. 1954.

This bulletin was written as a guide for prospective dairy farmers or for existing ones. To study the farm organization on farms where dairying is the major source of income, an analysis was made of the crop and livestock program, capital investment, labor use, expenses, and income on 49 Grade A farms as taken from farm record summaries in 1951.
R. W. Hunt

507. Increasing net returns on a Tennessee farm. T. J. WHATLEY, L. H. KELLER, and F. M. DEFRIESE, Tenn. Agr. Expt. Sta., Knoxville. Farm Econ. Cir. 3. 1954.

Directions are provided for operating and improving a 157 acre Grade A dairy-tobacco farm in the lower east Tennessee Valley.
R. W. Hunt

508. A comparison of the bulk and can systems for handling milk on farms. P. E. JOHNSON, H. C. OLSON, and R. L. VON GUNTEN, Okla Agr. Expt. Sta., Stillwater. Bull. B-436. 1954.

This study was limited to one dairy farm, however, certain advantages of the bulk handling system were observed. The two most attractive advantages were a saving in labor and the fact that the farmer sells his milk on the premises. In connection with the latter point, an average loss of milk per can was found to be 0.32 lb. Standard plate counts of both raw and pasteurized milk were lower for milk collected under the bulk tank system. Neither milk-handling system contributed to off flavors.
R. W. Hunt

509. Receptacle for milking apparatus. C. A. THOMAS (assignor to Babson Bros. Co.). U. S. Patent 2,706,965. 6 claims. Apr. 26, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 4: 449. 1955.

A milk reservoir for collecting milk from a mechanical milker, consisting of a bowl and lid. The lid is held tightly to the bowl by a clamp which is attached to an adjustable upright at the side of the cow. The size of the bowl and its distance from the floor is such that the teat cups when disengaged from the udder do not touch the floor. R. Whitaker

510. Milking apparatus. C. A. THOMAS (assignor to Babson Bros. Co.). U. S. Patent 2,709,416. 11 claims. May 31, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 5: 602. 1955.

Equipment is described for mechanical milking of a cow in a stall elevated at a level above the operator, including a frame for supporting a milking claw, and a milk can type of reservoir for accumulating the milk. R. Whitaker

ICE CREAM

511. Automatic batch weighing of mix ingredients. ANON. Milk Prod. J., **46**, 6: 20. 1955.

A simple and practical method for automatically weighing mix ingredients is described. This automatic weighing system is being used in the manufacture of liquid and powdered infant foods. J. J. Janzen

512. Texas mellorine volume jumped 49% in 1954. ANON. Ice Cream Trade J., **51**, 4: 32. 1955.

Competent observers estimate 1954 national production of mellorine somewhere between 40 and 50 million gal. Texas gallonage for 1954 was approximately 16.4 million gal., an increase of 49% over 1953. W. H. Martin

513. Exclusive analysis of 1954 packages by container sizes. ANON. Ice Cream Trade J., **51**, 4: 34. 1955.

Approximately 42% of the 750 million gal. of ice cream and related products produced in 1954 was marketed in factory-filled packages. About 25% of the total production was marketed in ½-gal. units. Some of the reasons advanced for the sensational climb of the ½-gal. size were economy, increased home freezer space, larger purchases, more outlets, larger families, convenience, and television, which tends to keep people at home, increasing overall demand for packaged ice cream.
W. H. Martin

514. What plant waste can mean in lost dollar-and-cents sales and profits. F. E. SMITH, Costa's Ice Cream Co., Woodbridge, N. J. Ice Cream Trade J., **51**, 4: 36. 1955.

Daily fat losses in an ice cream plant may be due to failure to check weights of incoming raw materials, inaccurate scales, mix lost due to failure to drain pipe liner, mixer running over formula specifications, and package overweight. Another source of waste comes from wasted cartons. These losses can be kept down to 1% or less by keeping adequate records which enable the production manager to know what losses are occurring each day.

W. H. Martin

515. Equipment for soft-serve stores. J. J. SHEURING, Univ. of Ga., Athens. Ice Cream Trade J., **51**, 4: 48. 1955.

The following guide should be used in the selection of freezers for soft-serve stores: (a) Will the freezer meet sanitary standards required by regulating official? (b) Is the cost of the freezer reasonable when compared with similar equipment? (c) Can the freezer be serviced and repaired with a minimum of labor and expense? (d) Is the freezer easy to clean and operate? (e) Will the freezer dispense efficiently and rapidly? (f) Should one large or two small freezers be purchased? (g) Has the freezer been accepted by the soft-freeze industry? (h) Does the manufacturer have a good reputation backed by years of sound business experience? (i) Does the freezer operate quietly with a minimum of control? (j) Will the freezer be easily operated by untrained personnel?

W. H. Martin

516. The relation of foam to the presence of coliform in pasteurized mix. H. B. SIEGMUND, Hendler Creamery Co., Inc., Baltimore, Md. Ice Cream Trade J., **51**, 5: 42. 1955.

The elimination of foam before heating the mix in the HTST heater appears desirable. Foam may pass the actuating thermostat in a flow diversion valve without being detected. Several systems employ a partial vacuum as a means of foam elimination to prevent burn-on in the heater and to overcome pasteurization deficiencies.

W. H. Martin

517. Soft-serve mellorine. E. M. DECK, Mrs. Tucker's Foods, Inc., Sherman, Texas. Ice Cream Trade J., **51**, 5: 40. 1955.

Formulas for mellorine-type of soft-serve mix containing 4 and 6% fat are presented. New types of fats which resist churn out in the freezer have been developed. Mono and diglyceride types of emulsifiers seem to work best with the new type of fat. The problem of off-flavor in mellorine-type products in some instances was found to occur when the freezer operator added some types of emulsifiers at the freezer.

W. H. Martin

518. Formulation of soft-serve mixes. J. J. SHEURING, Univ. of Ga., Athens. Ice Cream Trade J., **51**, 5: 22. 1955.

Factors which must be considered in the formulation of soft-serve mixes are legal standards, types of freezers that are used, cost of the mix, milk products available, competition and some particular problems of whipping and dryness of the product. If local and state ordinances permit, an excellent soft-serve mix may be made by using 4-6% fat, 11-13% snf, 13-15% sugar, 2-4% stabilizer and .10 to .20% emulsifier. The comparative nutritive value of soft-serve and ice cream frozen to different percentages of overrun is presented in tabular form.

W. H. Martin

519. Liquid sugar in ice cream. H. F. DEPEW, Natl. Dairy Prod. Co., New York, N. Y. Ice Cream Trade J., **51**, 5: 48. 1955.

Some of the advantages in the use of liquid sugar are: (1) improved sanitation, (2) saving of labor, (3) decreased warehouse costs, and (4) convenience. The USDA has prepared tentative proposals for U. S. standards of grades of liquid sugar, and hearings are being held. However, it may be some time before standards are adopted. Plants should check each shipment for color, flavor, and odor, per cent of sugar solids, appearance, bacterial count. The standard plate count should be less than 100 per g., yeast and mold, less than 10 per g.; and coliform count, less than 10 per g. Extraneous matter should be checked and a score of 25 or less by the Connecticut Standards of 1931 should be required.

W. H. Martin

520. Freezing of fruits for ice cream. S. D. PRICE, Chill-Ripe Fruit, Inc., New York, N. Y. Ice Cream Trade J., **51**, 5: 36. 1955.

Steps in the processing of fruit for ice cream include the field control of the raw product, rigid plant inspection, segregating of the fruit to meet individual industry requirements, and careful processing. Proper mixing of sugar and fruit is important. Sirups are undesirable for sweeteners, because of the water in the sirup, which softens the fruit. Opinions differ on the desirability of adding stabilizer to fruit. Instructions are given for processing peaches, citrus fruits, and other fruits.

W. H. Martin

521. Ice cream block marking device. G. A. STASINOS. U. S. Patent 2,710,450. 6 claims. June 14, 1955. Offic. Gaz. U. S. Pat. Office, **695**, 2: 183. 1955.

A taut wire device for slicing blocks of ice cream into rectangular shaped individual size portions.

R. Whitaker

522. Machine for applying dry coatings to confections. P. W. PARRY and J. G. MONTEITH. U. S. Patent 2,710,588. 13 claims. June 14, 1955. Offic. Gaz. U. S. Pat. Office, **695**, 2: 216. 1955.

Portions of ice cream frozen on a stick and suspended by the stick on the underside of a conveyor, pass through a spray of coating

material in order to produce a coated bar. The dried material is formed into a spray by a rotor to which a series of small buckets or cups is attached. The material is scooped up and discharged upward around the hanging ice cream bars in a continuous stream, the excess falling back to be resprayed. R. Whitaker

523. Ice cream packing equipment. G. G. ALEXANDER. U. S. Patent 2,708,055. 4 claims. May 10, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 2: 223. 1955.

A filler for filling brine tank or other molds, consisting of a hand-operated pump, a manifold, and flexible conduits for direct filling of a row of molds. R. Whitaker

MILK AND CREAM

524. Deionization of milk. E. G. STIMPSON (assignor to National Dairy Research Laboratories, Inc.). U. S. Patent 2,708,632. 28 claims. May 17, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 3: 384. 1955.

A mixed bed of about 1 part anion exchange resin and from 0.8 to 1.33 strongly acidic cation exchange resin is used to deionize milk. The pH of the milk is maintained within the range of 5 and 7. R. Whitaker

525. High protein deionized milk and process of making the same. E. G. STIMPSON and L. K. RIGGS (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,708,633. 37 claims. May 17, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 3: 384. 1955.

The deionized milk produced as described in Abstr. ‡524, is concentrated to a total solids content of at least 30%, the pH is adjusted to within the range of 5.8 to 6.2, and mixed with a protein such as casein. R. Whitaker

526. Bepaling van calcium en magnesium activiteit in melk en melk producten (Calcium and magnesium ion activity in raw and processed milk). A. VAN KREVELD and G. VAN MINNEN. Neth. Milk Dairy J., **9**, 1. 1955.

Ion activities in milk and milk products were determined by equilibrating the product with a cation exchanger (Duolite C 20). In raw milk the concentrations were 2.2 m*M* Ca^{++} and 0.8 m*M* Mg^{++} per l., compared to a total concentration of 30 m*M* Ca and 5 m*M* Mg per l. The results obtained with skimmilk indicate that separation did not greatly affect Ca^{++} and Mg^{++} activities. Pasteurization caused a marked reduction in the activities of the Ca and Mg ions. The Ca^{++} and Mg^{++} concentrations of 2 samples of reconstituted milk powder resembled those of pasteurized milk; one sample corresponded to raw milk. The ion exchange method applied to evaporated milk (8% fat, 18% SNF) showed *(a)* the condensing operation raised Ca^{++} and Mg^{++} activities, but less so than K^{+} and Na^{+}, *(b)* homogenization did not affect their activities, *(c)* sterilization lowered Ca^{++} and Mg^{++} activity. Addition of phosphate reduced Ca^{++} and Mg^{++} activity. W. C. van der Zant

527. How food ultrasonics are shaping up. R. MCL. WHITNEY, Univ. of Ill., Urbana. Food Eng., **27**, 5: 80. 1955.

Two key properties of ultrasonic waves which make them a useful tool for the food engineer are that they may be focused and can be controlled. In the food industry greatest interest lies in possible substitution of ultrasound for heat sterilization. At the Univ. of Ill., work on appropriate instruments and techniques has resulted in development of a suitable generator and tank assembly which is described. A main objective of the study has been to place bactericidal effects of ultra sound on a quantitative basis. Preliminary results appear promising.

Most work in the field of ultrasonics is still in the experimental stage, but various workers have developed applications that have been successful, at least on a laboratory scale. These include homogenization of milk, preparation of cream cheese mixes, prechurning of cream, dispersion of moisture in butter, and speeded ripening of cheese. There have been other applications with sugar, starch, hops, and grain. Some of the unsolved problems involved undesirable flavor changes, destruction of vitamins, and failure to inactivate some enzymes.

T. J. Claydon

528. What cathode rays do to eggs and milk. N. W. DESROSIER, F. J. MCARDLE, H. A. HOLLENDER, and W. W. MARION, Purdue Agr. Expt. Sta., Lafayette, Ind. Food Eng., **27**, 5: 78. 1955.

Products were treated in a linear accelerator developed at Purdue Univ. and modified to produce 100,000 rep per min. Inoculated fresh shell eggs could be sterilized with a radiation dose of 600,000 rep. However, a stale flavor was produced and the quality of the eggs was lowered. With raw skimmilk undesirable flavors resulted. There was an increase in rennet coagulation time and a decrease in casein heat stability. While cold sterilization offers interesting possibilities many problems remain to be solved. T. J. Claydon

529. Honolulu to Midway—Navy's longest milk run. A. GAETH, Dairymen's Association, Ltd., Honolulu, Hawaii. Milk Dealer, **44**, 8: 50. 1955.

Milk processed and packaged in paper cartons at Dairymen's Association, Ltd. in Honolulu, is packed into large insulated canvas bags holding 84 qt. Twice a week 1,000 qt. in these canvas containers are delivered to the Navy for transportation by Military Air Transport Service to Midway. The milk can be carried the 1,149 miles to Midway without souring.

C. J. Babcock

530. Een vereenvoudigde microbiologische methode voor het aantonen van conserveermiddelen in melk en melkproducten (A simplified microbiological test for the detection of preservatives in milk and milk products). D. A. A. Mossel, J. G. Mandersloot, and A. S. S. Gentis. Neth. Milk Dairy J., **9**, 63. 1955.

A sample of milk (50 ml.) is enriched with 2.5% glucose, inoculated with approx. 10^4 cells/ml. of bakers' yeast, and incubated in Einhorn tubes for 48 hr. at 25° C. The volume of gas formed in the closed part of the fermentation tube is compared with the volume of gas formed with a control milk. The common antibiotics used in veterinary therapeutics did not interfere. This test could detect $1.5 \times 10^{-3}\%$ H_2O_2 and 2 mg. Br per l. (ethyl monobromoacetate). Compounds yielding free chlorine could not be detected up to levels which are detectable organoleptically. W. C. van der Zant

531. Tetrazolium reduction test for quality control of milk. H. Laxminarayana and K. K. Iya. Indian J. Dairy Sci., **7**, 83. 1954.

The tetrazolium reduction test compared favorably with the methylene blue and resazurin tests in detecting milk samples of poor quality. The time necessary to reduce tetrazolium (1 ml. 0.1% soln. added to 10 ml. milk, incubated at 37° C.) to color shade T_1 proved satisfactory to class milk in 3 grades, good (> 4 hr.), fair (2-4 hr.), and poor (< 2 hr.). The T_1 color shade was obtained by adding 0.025 ml. of a soln. of equal mixtures of 0.5% saffranin and 0.2% methyl orange to 10 ml. of sterilized milk. The reduction of tetrazolium was not affected by leucocytes and the test does not appear useful to detect abnormal milk. No definite relationship could be established between the reduction of tetrazolium and the Eh of milk.

W. C. van der Zant

532. Process for retarding the development of oxidized flavor in milk and product. E. H. Freund and A. C. Herro (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,709,137. 16 claims. May 24, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 4: 1955.

The oxidized flavor in milk susceptible to the oxidized flavor is prevented by blending hot unhomogenized milk in a temperature range between 110° and 165° F. or cold raw unhomogenized milk below 60° F. with homogenized whole milk at the rate of between 5% and up to the maximum amount which will not increase the cream volume of the finished product. The pasteurized blend has the same cream volume as the original unhomogenized milk when pasteurized. R. Whitaker

533. Low-sodium milk and processes for producing same. A. L. Chaney and K. D. Johnson (assignor to A. L. Chaney). U. S. Patent 2,707,152. 8 claims. Apr. 26, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 4: 494. 1955.

Low-sodium milk for dietetic purposes is made by passing the milk through a bed of synthetic potassium ion exchange resin. By maintaining the normal pH of milk, the finished product has the same pH and calcium content as the untreated milk. R. Whitaker

534. Bottle for liquid dairy products. E. Sokolik. U. S. Patent 2,708,049. 1 claim. May 10, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 2: 221. 1955.

The top of this milk bottle is molded with two depressed grooves on the outside. The uppermost groove is utilized to hold the cap on the bottle, a lug or dog on the inside of the cover-all cap engaging this groove. The lower groove, similar in depth and shape, catches any drip which might run down the outside of the bottle when the contents are poured from the bottle. R. Whitaker

MILK SECRETION

535. Mammary gland studies. I. The identity and characterization of the smallest lobule unit in the udder of the dairy cow. A. F. Weber, R. L. Kitchell, and J. H. Sautter, Minn. Agr. Expt. Sta., St. Paul. Am. J. Vet. Research, **16**, 59: 255. 1955.

Serial sections from udders of 20 dairy cows examined in detail established the identity of the smallest secretory lobule units. Lobules from a parturient cow and one which had milked for 10 mo. were reconstructed in wax by tracing the outlines of the projected alveoli and ducts in dental wax plates, and assembling the models from the cut-outs. Excellent photographs of the models and photomicrographs of alveolar and duct elements are presented. The lobules were elongated and slightly flattened. In the lactating cow they averaged 1.5 mm. long by 0.9 mm. wide by 0.5 mm. thick. The total number of alveoli in 3 lobules were 156, 210, and 226, respectively. The average volume occupied by the lobules was 0.7 to 0.8 cu. mm. The intralobular duct system was simple and direct, with no consistent pattern of branching. The intralobular duct epithelium was very similar to alveolar epithelium and appeared to be secretory. Duct-alveolar combinations were often found in the periphery of the lobule. Otherwise no duct enlargements were noted in the lobule. E. W. Swanson

PHYSIOLOGY AND ENDOCRINOLOGY

536. Magenverdauung homogenisierter Milch beim Säüpling (Digestion of homogenized milk in the stomach of infants). A. Adam and E. Czech, Univ.-Kinderklinik Erlangen, Germany. Milchwissenschaft, **10**, 75. 1955.

Feeding trials were carried out on 18 infants, aged 6 weeks to 8 months with homogenized and nonhomogenized milk. In 15 infants the graphs of the sugar content in the stomach showed that homogenized milk utilized less digestive juice than did non-homogenized cow's milk. In

3 infants the graphs were atypical, which might be attributed to abnormal secretion. The difference of the dilution of the food in relation to the gastric juices is calculated as % and is presented in differential graphs. J. Tobias

537. The effect of feeding iodinated casein on yield and composition of milk. K. C. Sen, B. N. Premachandra, G. K. Murthy, N. N. Dastur, and D. Naryan. Indian J. Dairy Sci., **7**, 49. 1954.

Fourteen cows received 2.5 g. iodinated casein per 100 lb. body weight. The milk yield began to increase after 2 weeks; the maximum average increase was 14.3% during the sixth week of treatment. On withdrawal of the iodinated casein the yield returned to normal in about 7 weeks. With iodinated casein the average fat % increased from 4.53 to 5.15%. The total fat yield increased after 2 weeks of treatment, reached a maximum during the fourth week and then gradually declined. The S. N. F. of milk also increased during the treatment on an ave. of 5.6%. W. C. van der Zant

538. Longevity in Egyptian cattle and buffaloes. A. A. Asker, M. T. Ragab, and S. A. Hilmy. Indian J. Dairy Sci., **7**, 135. 1954.

Milk records comprising 520 lactations of 177 Egyptian cows and 484 lactations of 175 buffaloes were studied. The ave. productive life for cows was 3.5 lactations, in buffaloes 2.45 lactations. W. C. van der Zant

539. Relation of stage of lactation to volatile and unsaturated fatty acids of Egyptian cow and buffalo butterfat. M. M. Taha El-Katib. Indian J. Dairy Sci., **7**, 101. 1954.

Reichert (R), Polenske (P), and iodine (I) values were determined in butterfat samples of cows and buffaloes throughout the entire lactation period. The ave. R values of buffalo and cow butterfat were 29.18 and 25.14, resp. The R value of the buffalo butterfat was higher than that of the cow throughout the entire lactation period. The R values declined gradually during the lactation period. The P value of cow butterfat was higher than that of buffalo butterfat. The ave. I values of cow and buffalo butterfat were 33.01 and 32.1, resp. The iodine value increased as the lactation period advanced. W. C. van der Zant

540. Comparison of the metabolism of C^{14}-labeled lactose, galactose, glucose, and galactose in rats. F. J. Carelton, S. Misler, and H. R. Roberts, Natl. Dairy Research Lab., Oakdale, N .Y. J. Biol. Chem., **214**: 427. 1955.

When lactose, glucose, and galactose containing C^{14} was administered orally to rats, glucose was catabolized to CO_2 to a greater extent than galactose, lactose, or an equal mixture of glucose and galactose. Lactose and the glucose-galactose mixture were retained in the carcass to a greater extent than glucose or galactose administered individually. Galactose excretion via the kidneys was proportional to the dose administered.

When intravenous injections were made, glucose and galactose were quickly catabolized to respired air. Lactose was excreted primarily by way of the kidneys, and a smaller % of the administered radioactivity appeared in the respired air. B. L. Larson

SANITATION AND CLEANSING

541. Fly and insect control. Anon. Milk Prod. J., **46**, 6: 18. 1955.

Ways and means of protecting dairy cows from insects in the fields are discussed under the sub-headings of control sources, types of flies, horseflies, effective chemicals, and treatment methods. J. J. Janzen

542. Comparative cleaning by diphase cleaners and alkaline salts. L. Osipow, H. Pine, C. T. Snell, and F. D. Snell. Ind. Eng. Chem., **47**, 4: 845. 1955.

The detergent efficiency of alkaline salts was compared with that of a diphase cleaner for removing greasy soil of known composition from steel. The experimental soils were based on a mixture of mineral oil and petrolatum, with a small proportion of carbon black present as solid soil. Variations were made by adding different amounts of a fatty acid to the soil base.

Soils containing high levels of fatty acids would be expected to be most readily removed by alkaline salts because of soap formation. Nonetheless, the diphase cleaner was most effective in the removal of soil under all test conditions. The effectiveness of the alkaline cleaners rose rapidly with an increase in temperature, while the diphase cleaners maintained a high level of cleaning efficiency regardless of temperature. B. H. Webb

543. Waarde van de halamidverneveling voor de desinfectie van tanks (The value of an aerosol for the disinfection of tanks). G. Posthumus. Off. Orgaan van de F. N. Z., **46**, 851. 1954.

A 1% solution of chloramine (Halamid) in water mixed with a surface active agent was sprayed under pressure as very fine drops in a tank (100 ml. of solutions per m^3 tank volume). The aerosol remained in contact with the tank surface for 40 min. Application of a chloramine aerosol failed to be an adequate substitute for steaming. W. C. van der Zant

544. Washer for milking machines. R. E. Reeve. U. S. Patent 2,708,448. 3 claims. May 17, 1955. Offic. Gaz. U. S. Pat. Office, **694**, 3: 336. 1955.

An arrangement of a tank for holding a cleaning solution and a pipe system for cleaning the parts of a milking machine by circulation of the solution. R. Whitaker

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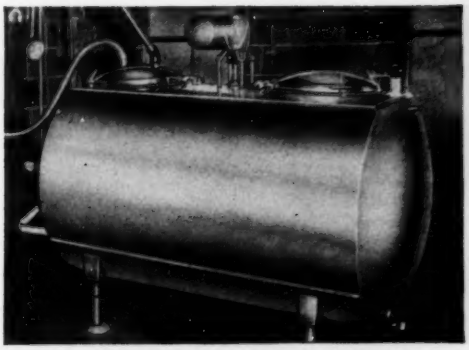

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